



### **Acknowledgments**

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**Figure 1. Friendly faces I met along the way.**

## **Introduction**

### ***My Background***

Since a very young age, I have been passionate about all things fishy and have kept a wide variety of fish species as a hobbyist. This passion included spending some 10 years breeding and showing fancy goldfish across the country for which I won many prizes. More recently, I have become interested in keeping fish in tanks that aim to mimic their natural habitats, thus enabling them to exhibit near-natural behaviours including spawning. I have also gained invaluable information into how fish live in the wild from various holidays I have undertaken, in particular snorkelling and fish collecting in the Amazon and various freshwater rivers and streams in Thailand where I observed a huge array of fish species, their assemblages and the diverse habitats in which they live. I have extended this passion for fish keeping into my work, and with an undergraduate degree, masters degree and PhD in fish related topics and I am now responsible for supporting a major fish research facility at the University of Exeter. Within this role, I am required to implement high specification fish husbandry on a variety of fish species to ensure the successful completion of research projects. The consideration of fish welfare in scientific research is a very recent phenomenon and the timing of the Winston Churchill Trust call for Travelling Fellowships in 'Animal Welfare' in 2008 could not have come at a more opportune moment for me. Therefore, with a combination of my passion for fish keeping, my current job overseeing a large aquarium facility and the difficulty in acquiring funding for such a project in fish welfare, I felt I had to grasp this wonderful opportunity with both hands and am very grateful that I was successful with my application.

### ***Fish in Science***

Although the use of animals in science always raises ethical concerns, there is no doubt that their use has been, and continues to be, crucial in bringing about many scientific discoveries and the use of fish in science, albeit less well publicised, is no exception. Fish are in fact the 3<sup>rd</sup> most widely used animals in scientific research after rats and mice and their uses range from helping our understanding of the genetic mechanisms that control how the human body works, in particular early life processes such as the development of the central nervous system, formation of vital organs (e.g. heart, eye) and tissues (e.g. muscular and vascular tissue) as well as studies on human health. Most notably, human health studies that include improving our understanding of how human diseases, ailments and/or developmental abnormalities arise and carrying out subsequent drug screening programmes in the hope to cure or relieve the symptoms of such conditions. It may seem strange that we can use fish and apply our findings to humans, but most vertebrates (animals with a spinal column) are very similar in their early stages of development. In fact, it is difficult to tell the difference between embryos from a human, a fish, and a chicken early in development. Because of these similarities, scientists have discovered that chemicals that affect a vertebrate animal embryo often have similar effects on a human embryo.



In addition to developmental and medical research, fish are also widely used as vertebrate model organisms in environmental monitoring programmes for assessing the effects of pollutants (e.g. sewage effluent), released by human activity, on the health of the environment including their potential impacts on other vertebrate species. In brief, and in the area of research I work in, fish provide appropriate models for testing chemical pollutants as there is a significant degree of conservation of basic aspects of their physiology with other vertebrates (including humans), in particular the hormone system with which many of these chemical pollutants interfere. For example, fish exposed to agricultural chemicals, which enter our freshwater systems via runoff, have been shown to have developmental and reproductive abnormalities including reduced fertility, whilst similarly the decline in fertility and the increased frequency of reproductive cancers observed in humans in some parts of the world have themselves been associated with exposure to agricultural chemicals, in particular pesticides. In addition to understanding the ecological impacts of these pollutants in the wild, laboratory based studies using fish are conducted to identify the particular chemicals involved, with a view to advising on permitted safe levels for release of these chemicals into the environment and/or prohibiting their future use should they be deemed hazardous to human health.

### ***Fish Welfare in Science***

Despite the fact that fish have been used in scientific research for many decades, it is only in the last few years that the scientific community has begun to consider the welfare of the fish kept within their own laboratories. Welfare, in particular environmental enrichment (the provision of stimuli which encourages an animal to display more natural behaviours despite being in a captive environment), has largely been deemed necessary only for so called 'higher organisms' in particular mammals. In contrast, fish have suffered from a long-held perception (both in the public and scientific communities) that, as so called 'lower organisms', they are less intelligent and, therefore, have minimal care requirements to satisfy their survival. This, combined with the fact that fish facilities in scientific laboratories have typically been run by more traditional technical staff as opposed to fish specialists/enthusiasts, has meant that some aspects of fish welfare have rarely been considered to date with most fish being housed in bare sterile tanks in research laboratories. However, as we understand more about the role the environment in which an animal lives and has grown up in plays in an individual's life history success and decision making in later life, and, importantly, that this may even apply to so called 'lower organisms' such as fish (based on recent research), we are beginning to see a shift in our thinking of how these animals should be housed in laboratories. The Home Office that regulates the use of animals in science in the UK has recognised this fact and are now pushing for fish welfare to be a higher priority in research laboratories. But arguably, and more importantly, it is further scientific evidence that is needed for the requirement of new and novel approaches to further improve fish welfare if we are to see a shift in the mindset of traditional scientists for them to implement improved welfare practices voluntarily.

### ***Aims of My Fellowship***

The aim of my proposed fellowship was to provide information to improve the welfare of fish, in particular zebrafish (*Danio rerio*), used in research laboratories in the UK, including my own facility here at the University of Exeter, and then try to communicate my findings to a wider audience worldwide. This was clearly an ambitious proposal and one I realised was probably beyond the scope of this fellowship alone. However, in order to undertake such a project, the first thing I wanted to do, and where my fellowship really opened the door for me, was to find out what and/or if anything was already being done to promote fish welfare in scientific laboratories outside of the UK given that I already had a feel for what was happening in this country through my work. In addition I wanted to look for information outside of the scientific community, something that as scientists we often shy away from, and with my background as a fish hobbyist I knew that we should be learning a great deal from groups who specialise in animal husbandry, notably aquarists. Therefore, with these thoughts in mind, I decided that for the first 4 week phase of my 8 week fellowship, the best place to head was the USA. I chose the USA because it has a wealth of world-class research institutes/organisations that are leading the field in highlighting the importance and initiating the promotion of good husbandry practices in laboratory-maintained fish. In addition, the USA is home to numerous public aquariums, many of which have specialist experience in, and carry out research into, improving fish husbandry practices, typically striving to mimic their natural habitats, promoting natural behaviours, and thus optimising animal welfare. At both types of organisations I wanted to spend time behind the scenes, interview key members of staff and find out about the husbandry practices they employ and the requirements that their regulatory bodies set for animal care/fish welfare.

For the second phase of my fellowship (again 4 weeks), I was particularly keen to see for myself how zebrafish, the most widely used fish species in science, and one that is key to our work at the University of Exeter lives in the wild. This unassuming little fish helming from the freshwaters of India and Bangladesh, with no commercial value and measuring only 3-4 cm long when fully grown, was propelled into the forefront of scientific research in the late 1960s by geneticist George Streisinger and colleagues. Known to fish hobbyists for over a century, the zebrafish has since yielded numerous and much needed insights into human biology, including the formation and function of individual tissues, organ systems and neural networks, and human disease mechanisms. More recently, the zebrafish has been used for studies into human health, for example neurological/neurodegenerative disorders, fear and anxiety, drug and alcohol addiction, cognition and learning, and ageing, as well as a model for studying animal behaviour and for numerous studies in (eco)toxicology. The use of the zebrafish in science is now so global that there is even a peer-reviewed journal devoted to and named 'Zebrafish', international conferences solely focused on work using zebrafish and tens of thousands of peer-reviewed papers/articles published utilising zebrafish across a wide range of scientific disciplines.

***Phase 1 of my fellowship; 4 weeks in the USA (04/11/08 – 02/12/08)***

**1) Boston 4<sup>th</sup> - 8<sup>th</sup> November**

First stop Boston, Massachusetts, home to the beloved Red Sox baseball team, and location for the acclaimed TV series Cheers, historical streets and beautiful autumnal foliage but, more importantly for me, home to the ZHA, the 'Zebrafish Husbandry Association', and the location for my first meeting on fish welfare. Arriving by coincidence on election night, the City was buzzing as Barack Obama was on the verge of being heralded into The White House but, unfortunately, jet lag and the prospect of a talk put pay to Amy and I joining any such celebrations for too long and it was an early night to recharge, but what a great start to the trip and a great time to be in The States!

We soon met up with Christian Lawrence, the ZHA Chairman-Elect and manager of the Zebrafish facility at the Children's Hospital, Boston, which provides embryos for research to one of the top paediatricians in the world, and Joe Sillitti from Techniplast who was being inaugurated as the new president for ZHA that day. Chris told me that the mission of the ZHA was 'to promote and improve husbandry standards for the usage of zebrafish in scientific research' and he was kind enough to invite me to speak at one of their regular meetings held at the Children's Hospital. Here I was joined, either in person and/or (rather dauntingly at first) live on-line by a number of ZHA members, who consult in zebrafish husbandry and run large and innovative zebrafish husbandry facilities. These included Amy Doherty from the Mass General Hospital Cardiovascular Research Center, as well as a number of key staff, for example Jason Best (chief technician of Chris's facility) and students who were working with zebrafish. I gave an hour long talk entitled 'Zebrafish Welfare and its Implications for Ecotoxicology', which included an introduction into my work at the University of Exeter (as described earlier), potential reasons supporting improving the welfare of zebrafish (scientific, ethical and government driven) and finished with an insight into the Winston Churchill Trust and the aims of my fellowship. The talk seemed to go down very well and I was inundated with questions afterwards. It soon became clear why Boston was the 'hub' for the ZHA with so many people involved in zebrafish research based here and so many incredibly large zebrafish facilities in such a relatively small area, some with thousands of tanks and requiring tens of staff to maintain.

Chris is probably the driving force for improving zebrafish welfare in the US and, since I returned from my visit, he has published an article compiling the opinions of various colleagues in the US on this area. The first realisation upon being shown around his zebrafish facility, and in contrast to mine, was the sheer number of transgenic and mutant strain zebrafish (literally hundreds) that are required for their area of medical research. As a consequence, his aquarium contained a vast number of small tanks, each holding a specific strain of zebrafish. These fish have to be bred routinely, as the majority of their research is conducted on their embryos. In order to breed these fish and collect the embryos, a sexed pair of fish (one male and one female) is captured from their stock tanks and placed into a separate small static tank overnight (or sometimes for 2 or 3 nights) to breed before being



returned to their original holding tanks. This process is then repeated regularly throughout their adult life, as and when embryos are required. In the aquarium I run here at Exeter, which is largely for ecotoxicology research, we predominantly use a single strain of zebrafish and need large numbers of adult fish for our research. As a result, our aquarium is run with far fewer, but much larger, tanks to hold our fish. We also require large numbers of embryos for our work but, because we can use larger tanks, we are able to place breeding chambers directly into our main stock tanks. The adult fish then spawn their eggs over the breeding chambers (a mesh lid large enough to let the eggs through but too small to allow the adults in prevents the eggs from being eaten) which can then be removed from the stock tanks when they are required and the eggs collected from them, with little or no disturbance to the adult fish.

It was clear from the start that our aquariums were set-up and run quite differently. This was largely to cater for our own specific research requirements which centred on the number of strains of fish necessary to support our particular research programs. However, there was one major commonality between our aquarium systems, and with other laboratory fish facilities I would see throughout this trip, and that was that our tanks were kept bare (with the exception of the breeding chambers we place in our stock tanks in the mornings) and appear quite sterile environments for the fish to live in. Chris told me that they weren't actually being asked by their regulatory bodies to consider adding enrichment (for example, through the addition of plants or substrate) to their tanks unlike what has recently been muted in the UK and is starting to gain some research interest. He told me that in the US every research facility is regulated at a local level by the Institutional Animal Care and Use Committee (IACUC) to ensure that all research conducted at the institution is in compliance with the Animal Welfare Act 1966 and the Public Health Service Policy on the Human Care and Use of Laboratory Animals. The IACUC must consist of a minimum of three persons; a Doctor of veterinary medicine, one member unaffiliated with the facility, and one member affiliated with the facility. All members must possess sufficient ability to assess animal care, treatment, and practices in experimental research as determined by the needs of the research facility and societies concerns regarding animal use in research. In addition many institutions undergo voluntary review by the Association for Assessment and Accreditation of Laboratory Care International (AALAC), which Chris said is considered the gold standard for animal programs. Here in the UK a similar procedure exists whereby each designated facility is also required to establish a committee and oversee animal work to ensure that all research conducted is in compliance with our own Animals (Scientific Procedures) Act 1986. This committee is often referred to as the Ethical Review Group and consists of; one member of academic staff from a school of the University that does not currently carry out research using animals under the Act, one named veterinary surgeon, one named animal care and welfare officer from each school that uses animals, two members of academic staff who carry out research using animals under



**Figure 2. Examples of the standard tanks and racking systems typically used to keep zebrafish in scientific research facilities in the United States. Top right picture shows an 'off the shelf' Aquatic Habitats System. Central picture shows a small plastic tank with plastic plant added and sloping base to create shallow edge used for traditional method of pair-wise breeding of zebrafish.**



the Act and the ERG secretary. The Home Office Inspector also has the right to attend any meeting of this group and has regular impromptu inspection visits. The ERG also review project and personal licence applications before being submitted to the Home Office.

Carrying on with our tour, it was also immediately obvious from the first moment I saw the size and number of tanks Chris manages in his zebrafish facility that the idea of being able to improve fish welfare through the use of enrichment would be a very difficult task for him, much more so than in my aquarium for instance. In fact, as I discussed with Chris, adding enrichment to such small tanks could even be prohibitive to the welfare of the zebrafish as it would become difficult to monitor the fish easily for health checks and/or catch them when required, potentially even increasing stress. From a practical perspective, there would also be a big increase in cleaning with so many tanks involved, as well as the additional cost implications of enrichment material and extra labour. This was also true of a second large zebrafish facility also at the Children's Hospital run for Leonard Zon that Chris and Isaac Adatto, the chief technician showed us around. This facility again had vast numbers of very small tanks. However, Chris did point out that they had indeed made some modifications to their small static breeding tanks which may be classed as enrichment. Instead of a bare tank, they now incorporate a piece of plastic plant to act both as a stimulus for breeding and as a refuge if one fish wants to avoid harassment from the other. They also now incorporate a sloping base in the tanks to mimic the shallow edges in which zebrafish breed in the wild. Chris told me that just these relatively small changes to the static breeding tanks had resulted in an increase in egg production. This is good for their research but, more importantly, has improved fish welfare by reducing fish deaths that sometimes occur through harassment/stress as zebrafish can be aggressive towards each other when kept in pairs, even if only for a short period of time. Chris told me that each strain of zebrafish they maintain is very valuable to them. This is because they have often taken many generations and crosses to develop and sometimes at considerable cost. It is, therefore, clearly in their best interest to keep the fish in good health and keep them breeding for as long as possible.

In their main holding/stock tanks though, instead of enrichment per se, their focus from a fish welfare perspective is on providing the best possible water quality via high specification filtration equipment, provision of high quality food at every stage of zebrafish development to promote good growth, health and breeding condition, and prevention of disease through good husbandry practices and training of staff in fish care. This, needless to say, should be the first priority of any fish facility in any laboratory and is something we also pride ourselves in achieving here at Exeter. However, Chris had some concerns that with such a rapid expansion of the zebrafish 'model', particularly into laboratories that are only used to handling mammalian species, there could be a real lag between the time they start using zebrafish and them becoming well versed in the biology of the species to be able to care for them correctly. At the same time, Chris was also concerned at how the regulators themselves would be able to set out appropriate regulatory guidelines for zebrafish care given that they are typically much less familiar with zebrafish biology than they are for mammalian species. It was interesting that the



concerns Chris raised about the future of zebrafish welfare in the US were also concerns I shared here in the UK especially as the use of the zebrafish has also grown exponentially here as well as across mainland Europe and in all areas of scientific research but without necessarily the concurrent increase in trained personnel and guidelines to care for them correctly. Chris's enthusiasm and knowledge had provided a great start to my trip and it was great to see how active the ZHA is which can only be good for the welfare of zebrafish and fish in general in the US. Unfortunately, it was time for us to leave Boston, following a quick stop at Boston Aquarium, and although sad to go, I was enthused and positive for what was to come for the rest of my trip.

## **2) Cape Cod 8th-11th November**

Amy and I headed a short distance down the East coast of America to Cape Cod, staying at the Woods Hole Passage Inn, a century-old carriage house set amongst beautiful woodlands that served traditional hearty American fare for breakfast. A daily 3-mile walk through the woods in their full autumnal colours and along the ocean beaches took us to Woods Hole where the world famous Oceanographic Institute (WHOI), known for discovering the wreck of RMS Titanic no less, and the Marine Biological Laboratory (MBL) are located and destinations of my next meetings with fishy friends. Here, I met up with post doc researcher Jed Goldstein and fish facility manager Bruce Woodin of Professor John Stegeman's toxicology group at WHOI and Bill Mebane (marine resources centre operator) at the MBL for a tour of their facilities.

The zebrafish facilities at WHOI were a standard racking system produced by Aquatic Habitats, one of a number of companies that produce 'stand alone' fish keeping systems for scientific laboratories. The tanks were similar to Chris's in Boston, in that they were relatively small bare tanks, racked a number of tiers high. However, unlike Chris's, these came with their own built-in filtration systems and can be bought 'off the shelf' as one complete unit. For this reason, this type of system is now commonplace throughout zebrafish facilities worldwide, particularly in research laboratories that are required to maintain lots of strains and therefore require lots of tanks. These 'off the shelf' systems do allow a couple of different size tanks to be used and the larger tanks did actually permit breeding chambers to be placed directly into them so that zebrafish embryos could be collected without removing the adults to separate breeding tanks (as discussed previously). Bruce at WHOI told me that he preferred this option of embryo collection because he had experienced problems in the past using the traditional pairwise breeding method in separate static tanks. He felt that the stress of regular fish handling (catching and moving fish between tanks) decreased the breeding success and breeding longevity in adult zebrafish. Also, and as observed by Chris in Boston, he mentioned that aggression between pairs of zebrafish sometimes resulted in fish deaths. However, Bruce also pointed out that from a practical point of view, although he agreed that the breeding chambers in the tanks provided some form of enrichment to the fish, they didn't want them in their tanks all of the time. In fact, only really when they needed fish embryos. If left in the tanks permanently, the fish would breed every day and so valuable embryos may be wasted. This was an interesting

point of view and shows that some balance may be required to satisfy the needs of the researcher and the potential welfare needs of the fish. In general though, it was still clear that providing any form of permanent environmental enrichment in these types of tank systems would be again difficult due to their small size as well as the limited access between each tier. Also, from a functional point of view, enrichment might actually compromise the efficiency of the filtration system given that it has been designed based on the tanks being used bare.

Bruce and Jed also raised the point that most laboratory bred fish have never seen anything other than a bare tank and so wondered about the benefits of enrichment if they have never seen it before (i.e. they don't know what they are missing or they might even be scared of it?). This is a question that has been put to me and other researchers that are pro-enrichment before, and one for which there currently isn't any hard scientific evidence arguing either way. However, the few studies that have investigated the role of environmental enrichment have certainly shown (in some fish species) that it can help reduce aggressive behaviour, increase foraging performance (important for finding food), and stimulate brain development. From a personal perspective, I have also witnessed a significant difference in the behaviour of zebrafish kept in bare tanks, which often show fairly repetitive swimming patterns compared to those kept in enriched environments, which show far more interactive behaviour and even from zebrafish that are placed in enriched environments having only ever experienced bare tanks before. Investigations of this nature have still not been thoroughly conducted. However, from some of the early evidence, further research is certainly warranted to address this issue. This is an area of research I hope to explore myself in the future with the knowledge gained from my fellowship.

One important feature that the racking system they employed had and that, although not necessarily environmental enrichment, was certainly benefitting the welfare of the fish, was the use of coloured plastic lids and backings to the tanks to reduce any reflection. Reflective surfaces are a common problem in bare tanks and are exasperated in open shelf racking systems creating potentially very stressful conditions for the fish, particularly given that all surfaces tend to be reflective including the bottom of the tank. Anyone, for example, working on one row of tanks is likely to disturb the row of fish above and/or below them and this is likely to be stressful for the tank inhabitants. Some strains of zebrafish, and/or other fish species, used to confinement in tanks may adapt better and quicker to this scenario whilst other strains. In particular wild-type strains and fish that are not used to tank confinement, will no doubt suffer stress every time tank maintenance occurs.

Following these discussions I then gave my talk for the second time to Professor Stegeman's research group at WHOI and they were all intrigued (as in my first talk) to learn about my Winston Churchill Fellowship, the trust itself, and whether they could apply for a travel fellowship themselves! Following again another positive response from my talk, I then made the short walk down to the MBL to meet Bill Mebane (who had already listened to my talk online!) and his zebrafish and cuttlefish facilities. Funnily enough, the fact that I came all of the way from the UK became an excuse for Jed and Bill to meet each other for the first time, despite them working within a few hundred yards

of each other for many years and may be the start of future collaborations between them!

I could immediately tell that Bill was very much pro-enrichment for zebrafish and he told me he felt that zebrafish husbandry was still poorly understood from this respect. He was currently running a small unit for zebrafish using the same 'off the shelf' racking system as WHOI from Aquatic Habitats but he felt that, despite their popularity and efficiency for space and provision of good water quality, that the small tank size was not conducive to adding enrichment. Bill even went further to suggest that these types of tank system may even restrict a fish's movement and potentially their ability to grow at a normal rate. Interestingly, Bill showed me a new tank system that he was testing out, also manufactured by Aquatic Habitats and called the Mass Embryo Production System (MEPS). The first really interesting thing about this tank was its size; considerably larger than the tanks on the 'off the shelf' racking system and, therefore, capable of holding many hundreds of adult fish but still allowing them lots of space to move freely. It contained a large funnel, covered with a mesh and multiple breeding sites with plastic plants attached to each site. The funnel sat just below the surface of the water to mimic the shallow weedy areas that zebrafish use for breeding in the wild and any eggs spawned over the funnel fell through the mesh and were captured in a cup for easy collection. This system not only promoted enrichment but the automatic embryo collection feature eliminated the need to handle adult zebrafish as it was no longer necessary to transfer them into separate breeding tanks. Bill felt that the continual handling of zebrafish from holding tank to breeding tank was an important welfare issue and he felt that over handling caused too much stress to the fish, but with this new system this handling stress on the fish is reduced/removed. Bill was also testing out different types of plastic plant on this setup, at the time of my visit, to see if the fish had a preference for a particular type. At Exeter, we have found that zebrafish have a preference for, and spawn more eggs over, plastic plants that mimic long grass in shape. Since my return from the US, this new MEPS system has become commercially available from Aquatic Habitats and so it will be interesting to find out if it becomes popular within scientific laboratories. One downside, however, is that you couldn't have one of these tanks for all of the different strains of zebrafish many laboratories require for their research, because of insufficient space and prohibitive costs, and so it was probably only applicable to laboratories that used mainly one zebrafish strain. Interestingly, Bill was also caring for cuttlefish within his facility at the MBL. Cuttlefish (despite being an invertebrate) are regarded as highly intelligent organisms in the scientific community, and so Bill was housing them in tanks that contained a lot of enrichment to keep them occupied.

Bill took a phone call.....his wife wondering where he was....., we had been chatting so long we didn't realise it was 7pm and time for us to leave and head back for one last night in the B&B followed by one last hearty American-style breakfast (waffles and maple syrup!) and then.....



**3) Atlanta and Tennessee via a whistle stop tour of New York City**  
**11th-16th November**

Next stop, and a break from research laboratories, was a visit to Atlanta and Georgia Aquarium, home to whale sharks and the current location of the world's largest aquarium (see front cover). But first, and as it was almost on the way, a quick whistle-stop tour of New York City was a must. Despite arriving late in the evening, a stroll down Broadway (spotting Robert DeNiro, Julia Stiles, and Deborah Messing [I think! and, if correct, the woman who plays 'Grace' in 'Will and Grace'] at a James Bond film premiere), through Times Square and then up the Empire State Building at close to midnight to admire the stunning views quickly gave Amy and I a feel for why New York is often referred to as 'The City that Never Sleeps'. The place was a hive of activity even at this late hour, when most English Cities would be long put to bed! The next day, and our only full day in the City, meant an early start and breakfast in the beautiful surroundings of Central Park. This was followed by a walk around lower Manhattan to see 'Ground Zero' the 9/11 site, Wall Street, and Battery Park and a boat trip around New York harbour to take in the Statue of Liberty and Brooklyn Bridge. We finished it all off with dinner in Ellen's Stardust Diner located on Broadway where the waiters and waitresses sing to you whilst you eat, at first a bit embarrassing for a couple of prudish Brits, but once in the swing of it....!

Following an 18-hour overnight Amtrak train from NYC, we arrived in Atlanta, Georgia, dumped our luggage and headed straight for Georgia Aquarium with the excitement of seeing beluga whales and whale sharks, some of the largest animals ever held in captivity. Unfortunately, out of the 3 aquariums where I had tried to arrange meetings with key members of staff, Georgia Aquarium was the only aquarium where I was unable to meet anyone to discuss the fish welfare and enrichment practices they were employing for their exhibits. This could have possibly been because not long before my visit they had received bad press about the welfare of their whale sharks. Anyway, we continued on with our own private tour! It was clear that the promise of huge whale sharks in the largest tank in the world drew people in from miles around and you couldn't fail to be in awe of such a tank (front cover). However, with the exception of one or two tanks, in particular one tank that mimicked a local coastal fish habitat and that contained sheepshead minnows, a commonly used fish species in laboratories that is rarely seen in its natural surroundings, Georgia Aquarium left me feeling slightly cold. I found that it was set up more like a theme park than somewhere for the pure fish enthusiast such as myself to go and it was a shame I could not speak to any staff to tell me otherwise!

It was then another early start the next day to get from Atlanta to Chattanooga and Tennessee Aquarium, home not to the world's largest tank but, more importantly for me and more relevant for zebrafish, home to the world's largest freshwater aquarium. Here we were met by assistant curator of fishes Carole Farmer. Tennessee is a much less well publicised aquarium than Atlanta. However, I found it to be far more educational and it certainly felt like the welfare of the fish came before the enjoyment of the paying public. Carole showed us around and talked at length about the different exhibits and the



**Figure 3. Examples of animal enrichment observed in public aquaria in the US. For example, top left and bottom right: tanks designed to replicate fast flowing stream conditions (Tennessee), Top right: fully planted tank designed to replicate a tropical freshwater habitat, Middle right; giant kelp tank replicating local marine coastline (Monterey).**



types of husbandry and enrichment programs they were running. In stark contrast to the fish in scientific institutions, these fish had every kind of enrichment imaginable in their tanks. These ranged from physical objects such as substrate (e.g. sand, gravel, shells), real and plastic plants, wood and rocks to the use of water currents and jets, and were all in an attempt to offer their exhibits a more natural setting and something close to what they would experience in the wild.

Carole told me she was very keen that the fish displayed as near natural behaviours as possible. She pointed out that, in order to achieve this, many of her exhibits were kept at a lower stocking density than many other public aquaria. This was again to replicate more natural conditions for the exhibits and is far removed from the high stocking densities and/or cramped conditions fish are often maintained at in laboratories. Carole said she had to compromise slightly to ensure there were enough fish to make a good exhibit for the paying public. But, in fact, I felt the public benefited more from this approach as they got to observe many of the fish displaying much more avidly with each other than normal. In addition, and probably a direct consequence of the added space the fish were permitted, many of the fish were even breeding (including some difficult to keep species). This was a sure sign that the fish were in good health and something the paying public may not witness so much in other aquariums where fish densities are kept elevated just to create an immediate, but nevertheless short-term, impact.

Interestingly, Carole also pointed out how understanding the biology of the fish and replicating some aspects of their natural habitat can actually be vital for the health of the fish. For example, Carole showed me to a tank of fish, commonly known as darters (due to their streamline shape and speed) that require the provision of water currents to mimic the fast flowing streams they occupy in the wild. Carole pointed out that without such water currents they would suffer a build up of fatty tissues. This may then have knock-on effects on the health of the fish as they get older. In addition, without currents, they certainly wouldn't display natural behaviours. Some fish species even need enrichment to carry out natural behaviours such as the provision of shells for shell-dwelling cichlid species to breed.

Carole was also keen to mention how lighting could play a role in the wellbeing of the fish. For example, some exhibits such as the salmon and trout tanks were able to have natural lighting (something that would be ideal for all exhibits). However, most tanks have, unsurprisingly, to be lit artificially and not only that but lit bright enough such that they can be viewed easily by the public. As a result, Carole suggested that the provision of enrichment in the tanks was also important in ensuring that there was always an area of refuge so that individual fish could move to avoid the light if they so wished. Interestingly, scientific fish facilities are actually very good at providing lighting regimes that mimic natural conditions including simulating dawn and dusk. This is important for preventing the stressful situation that occurs in most home aquaria of fish being plunged into either complete darkness at night time or bright light in the morning. Simulating dawn is also beneficial to encourage breeding in many fish species, including zebrafish, so actually replicating natural lighting regimes also has practical benefits. This is particularly so in zebrafish laboratories that require the production of lots of



embryos for their research. However, irrespective of whether enrichment is beneficial or not for laboratory fish, very little consideration has been given to the fact that the use of completely bare tanks provides no refuge for the fish away from bright light. Being subjected to almost permanent light could in itself be stressful to some fish species, and particularly if the wrong type of lighting (for example, the wrong wavelength and/or too bright a light) has been fitted in the first place.

Carole told me that inevitably, although she promotes the welfare of her exhibits as a priority, some compromises have to be made to meet the public needs and those of her directors as well as what is sometimes practical and feasible in what is essentially a profit-making business. For example, some of the larger fish exhibits didn't have fine sand as a substrate to match the situation in nature but had coarser gravel and/or rocks because the fine sand can find its way into the filtration systems and cause significant damage. This wasn't a problem for the fish in the exhibits Carole showed me but I had seen, and would subsequently see, other exhibits on my visit (not at Tennessee) where fish were being kept in tanks with inappropriate substrates. For example, I observed *Corydoras* species (a small Amazonian catfish) being kept on coarse gravel, instead of fine sand. This had resulted in a loss of their delicate barbels and meant that they were struggling to feed normally. Worse still, they were showing signs of infection that if left untreated would ultimately result in their death. Carole then pointed out that the rocks in the displays, particularly the larger exhibits, were often pre-fabricated concrete due to the prohibitive cost of the real thing and/or restrictions of collecting from the wild. However, this fabrication process has improved dramatically and often it was hard to tell if the rocks were real or not and certainly didn't seem an issue for the fish!

One of the major compromises she explained was the feeding regime. Feeding time is clearly a popular time for the public and, as such, is pushed by the directors of the aquarium as a way of getting more 'people through the gate'. This meant, however, that some of the exhibits were fed slightly more often than she would have otherwise liked. Carole felt that this overfeeding may have actually contributed to the decline in health and subsequent death of some of their conger eels (large marine species). Carole pointed out that this was more of a problem for the larger fish exhibits that people like to watch feed. Interestingly, for this same reason, exhibits such as the octopus were also being fed daily instead of perhaps only 2-3 times per week which is more akin to their natural feeding habits. She monitored this situation very closely and liaised with her directors to ensure that the fish/animal welfare was the first priority. In fact, in most cases I could see that they went to great efforts to feed both a good variety of food and the appropriate nature of that food (e.g. size, nutritional content) for each type of fish species.

Food can in itself be classed as enrichment for the exhibits and is clearly vital for their long term health and can also promote natural behaviours of the exhibits. Carole pointed to a tank containing red collar sunfish which she said had been looking in poor condition and showing little activity. She said they decided to try to source some of their natural food stuffs, in this case clams, to see if that would 'perk them up' a bit and improve their general condition. They responded rapidly to this change to their natural diet. Within a

very short time of feeding them clams, the sun fish's health improved dramatically and they even started displaying more natural behaviours. She said there is always a compromise when it comes to sourcing food for their exhibits because they are always having to compromise between the cost of the food, which is one of the most expensive outlays for a public aquarium, and the welfare of the fish. This clearly shows though that feeding the correct food from the start works and probably in the long term saves them money, as people don't want to pay to come and see fish in poor condition. Also, the aquarium isn't then lumbered with an exhibit they have to change, or fish they have to treat for health related problems caused by their poor diet. Carole then went on to point out some exhibits where they target fed certain fish species (e.g. paddlefish) to make sure they received the amount of food they required to keep them in good condition. This was especially the case in mixed species exhibits where she said that some fish can be greedy/aggressive at feeding time and monopolise all of the food at the expense of timid species/individuals.

Finally, Carole took us on a quick tour behind the scenes and her quarantine facilities. These were, in fact, all bare tanks, just like you would find in a fish laboratory facility but here they were like this for a number of reasons. For example, enrichment in tanks can actually prevent chemical treatments working effectively as they may absorb some of the chemical treatment or react with it. Additionally, a bare/sterile tank also prevents the build up of any bacteria and/or other fish pathogens that enrichment products can harbour. Clearly, if a fish is in a 'hospital tank', it is likely that it already has a compromised immune system and so the more sterile the conditions are during treatment the quicker the fish is likely to recover and be returned to its exhibit. However, fish under quarantine are typically only housed temporarily in such sterile conditions before being returned to the main exhibits, unlike for 'life' in a laboratory setting. During the fish's time in quarantine, Carole said that they conduct routine tests on the water and fish faecal matter, analysing for bacterial infections and other pathogens and, in newly acquired fish from the wild or donated from other aquarium facilities, for parasites (maybe through skin scrapes and microscopy) to ensure there is no transmission of diseases to the main display tanks. Suddenly, that was the end of an amazingly informative tour with Carole around her aquarium and one that would definitely be remembered by me with fondness and as one of the best public aquaria I have visited to date. It was now time for Amy and I to head further down the east coast to the comparative warmth (turned out they were having an un-seasonally cold spell!) of Florida.

#### **4) Florida 16<sup>th</sup>-23<sup>rd</sup> November**

We were staying in Tampa Bay, Florida, for this part of the trip and, on the drive in towards our hotel, we had already seen dolphins out of the car window playing close in to shore, pelicans dive-bombing into the sea to catch their food and behind every fisherman there always seemed to be a heron or two waiting patiently in the hope of a chance to grab an easy meal. Florida was alive with wildlife and Amy and I immediately knew we were going to

enjoy our few days in this part of the US. Actually, I had already been especially looking forward to this part of the trip anyway, because Florida is 'home' to the US ornamental fish farm industry producing as much as 95 percent of the entire US market for aquarium fish and plants for the hobby industry with an estimated value to the economy of \$50 million for the freshwater fish alone. One might say Florida was the perfect place for someone who spent much of his childhood keeping as many different types of fish as his parents and their house would allow - I was in heaven! Containing my excitement for a moment from seeing so many of the fish species I have kept over the years, and so many that I have wanted to keep, we headed first to visit Eric Herbst, Research and Development Manager for Aquatic Habitats (a division of Aquatic Ecosystems) in Apopka near Orlando.

Aquatic Habitats was founded in 1997 to meet the unique needs of the aquatic research community and is responsible for the 'off the shelf' zebrafish racking systems I had previously seen at WHOI and the MBL in Cape Cod which are now used by fish research laboratories worldwide. Interestingly Eric, had listened online to my first talk back in Boston (I even recall him asking me a few questions!) and fortunately listening to me hadn't put him off inviting me to come and see his company's main test facility whilst I was in Florida. Eric gave me a detailed appraisal of their zebrafish racking system which he had running in one of their test rooms and, having never used one of their systems before, this was very informative and extremely useful for me to see how it operated. There is no doubt that the Aquatic Habitat systems are extremely proficient at maximising tank numbers in a very small space. This, combined with a built in filtration unit that makes them completely self contained and their easy to use 'off the shelf' application, have to be major reasons as to why they have become so widely used, particularly across the US. Eric told me that Aquatic Habitats can also custom design systems as well and, in fact, can cater for almost all aquatic needs for any fish or aquatic animal species (for example, Eric also showed me a system for *Xenopus*, a commonly used aquatic frog in scientific research). Eric knew that, because of my background as a fish hobbyist/enthusiast, I was very much pro-enrichment in fish tanks. However, despite me questioning whether the Aquatic Habitats system does in fact provide enough space to allow for enrichment to be added to their tanks, I was left in no-doubt following Eric's demonstration that these systems provide optimal water quality for the fish. This is arguably the first and most important aspect of fish welfare and should be the starting point for all fish facility managers. Eric did also point out that each of the tanks on their racking system could, in fact, be removed and sterilised at high temperatures. This was also an important feature for fish welfare because it had been proven as an effective way to prevent disease and something I would learn more about when I reached Oregon in a few days time.

Eric then went on to show me Aquatic Habitats new 'MEPS' zebrafish system which I had already been privy to (and have previously discussed) when I met Bill Mebane at the MBL in Woods Hole. I certainly liked the look of this system and would consider using it here at Exeter because it provides both a very efficient way of egg collection with little or no disturbance to the adult fish and habitat enrichment through its use of plastic plants to stimulate breeding. The tank also comes in two sizes both considerably larger than



standard zebrafish tanks and this extra space is likely to promote more natural behaviours. In addition, the tank is not transparent (unlike plastic or glass tanks) and thus has no reflective qualities. As discussed previously, these can be very stressful to the fish. Apart from the downside of a high cost (approximately \$8,000 - \$15,000 each, for the small and large versions respectively) of these tanks, which I could see being prohibitive to them being as popular as some simpler alternatives, it was pleasing to see that Aquatic Habitats, through its research and development programs, were coming up with new and novel systems to meet both the requirements of the user (scientists in this instance) and the welfare of the fish.

Eric was extremely interesting to chat to and it was great getting the views of someone directly involved in the design of these systems. I also found it extremely encouraging that there seemed to be so much freedom of communication between the people I had met so far on this trip. Also, that everyone in the industry seemed enthused to promote good fish husbandry practice, with Eric no exception. Eric then, like so many people I had met on the way, couldn't let us leave without recommending a couple of places to visit to take in some of the wildlife of the area. It was already nearly 5pm but he suggested to Amy and I that we popped a few kilometres up the road to Wekiwa Springs to catch a bit of wildlife before the sun went down. We did just that and, although at first we thought we were going to be disappointed and not see anything apart from a scantily-clad glamour model who happened to be having a photo-shoot right where the wildlife should be, we were finally rewarded in the last 15 minutes before sunset. Here, in the peace and quiet (and no glamour model!), we suddenly spotted alligators, terrapins, longnose gar (large predatory fish), egrets and several other fish species including some non-native fish that should have been much further south in the Amazon! That was an amazing 15 minutes though and, combined with the hilarity of later telling Eric about the glamour model almost scuppering any chance of seeing wildlife, it was well worth the short trip. What it had also taught us was that Eric clearly knew where to go to see some of Florida's best wildlife and so his second recommendation of snorkelling with manatees couldn't be missed. But, first, it was time for my long awaited chance to visit some of Florida's ornamental fish farms.

We headed from our hotel for a short drive (by US standards anyway!) down to Ruskin where I had arranged to meet up with Dr Roy Yanong, Associate Professor and aquatic veterinarian at the University of Florida Tropical Aquaculture Laboratory (TAL), which is part of the Universities School of Forest Resources and Conservation (SFRC) Program in Fisheries and Aquatic Sciences. It was clear from the start that Roy was a real character but more importantly he was as equally enthusiastic about fish as me. Although we didn't have time to visit all of the 200 odd fish farms in the area to see the 800 plus varieties of freshwater fish they farm (something I would have been more than happy to do), Roy was kind enough to give up a significant amount of his time to give me a flavour of this industry as well as that of some of the work undertaken by his own laboratory. First of all, Roy gave us a tour of his own facilities at TAL which, established in 1966, has a specific mission to enhance the understanding of tropical, ornamental



**Figure 4. The Mass Embryo Production System (MEPS) developed by Aquatic Habitats and being tested at the time of my visit by Bill Mebane (MBL, Woods Hole) and Eric Herbst (Aquatic Ecosystems, Florida). Top left showing larger version, top right showing smaller version. Fish spawn over funnel, stimulated by plastic plant spawning sites. The tanks were large enough to allow natural group spawning by zebrafish.**

aquaculture through research and education. Roy and his colleagues work collaboratively to promote the advancement of aquatic animal medicine and fish health management through courses, work/study placements and scientific publications. They have a very important role to play, given the size and importance of this industry to the economy in Florida, as any losses caused by disease in Florida's tropical fish industry can, and have had, major economic impact to the area. They also have links to, and can help give advice to, fish facilities in scientific laboratories. Looking around their aquaria, they had a number of unusual freshwater fish species that are popular in the pet fish industry (for example, knife fish, puffer fish and archer fish). Roy told me that they were actually trying to improve their understanding on the biology of these species and in particular attempt to breed them as they were species that, until now, had proved either notoriously difficult to breed naturally or had never been bred in captivity. These attempts involved their researchers testing out different types of enrichment, water quality parameters, and food to try to breed these species and, once/if they established an effective method, they could then impart this knowledge to the fish farmers – something they had just been successful in achieving for a species of freshwater pufferfish. Roy told me that any husbandry information they could provide to the fish farmers trying to breed a new and/or difficult species of fish that they hadn't kept before would also significantly benefit the welfare of these fish, as the farmers wouldn't be starting from scratch, so to speak.

Following an entertaining lunch with Roy and Jeff Hill (one of his colleagues at TAL who was working on the impact of non-native fish species in the area), where Roy pretended to the waitress that it was my birthday to secure me a free cake (and it worked, I later found out a common trick of his!) we set off for a tour of two ornamental fish farms (one in Lakeland and one in Plant City). Here, we saw how they went about the mass production of fish, including zebrafish, for the hobby industry. The scale of these farms was amazing and it was interesting to see how they managed the huge variety of species they were breeding. The stock fish and any new offspring were generally being kept in large holding tanks/ponds much larger than anything we see in laboratory facilities. Given that most fish produced for the pet fish industry are small (typically less than 10 cm long with many popular species actually smaller or similar in size to zebrafish), this meant that they benefitted from having a lot more space to swim in than their laboratory counterparts. Also, the stock/growing-on ponds were under poly-tunnel style housing. This meant that the fish benefitted from natural daylight, which is much less stressful than bright artificial lighting. Algae was also allowed to adorn the sides and bottom of the tanks/ponds, and so their fish weren't being subjected to the stresses of reflective and/or transparent surfaces that we see in laboratories. Interestingly though, many of the fish species we saw being bred on the fish farms (for example, many species of popular tetras such as neons, cardinals and glowlights) were being bred using the same pair-wise breeding method that we had already seen used for laboratory-kept zebrafish and that we had discussed in terms of causing stress to the fish as a result of over-handling and/or aggressive behaviour. However, many of the fish species being bred here wouldn't actually spawn without some artificial or real plant/substrate. As a result, there was always something (funnily enough



Christmas tinsel worked well on one farm) in the tank to act as both a stimulus to encourage spawning and provide a refuge in case of harassment. Again though, this enrichment is only being used for a temporary period until the fish have bred and, once accomplished, the fish are put back into their bare stock tanks. So, despite many of the positives I saw in the holding tanks for these fish compared to tanks in laboratories, and having not mentioned the great lengths they went to provide good water quality, you couldn't change the fact that these farms are a business and, therefore, their main concern is always going to be about producing lots of fish cheaply. As a result, and aside from perhaps some of their most valuable stock fish, environmental enrichment is never going to be a big priority for these fish farms. Importantly, however, many aspects of fish welfare have improved in this industry which used to be plagued by poor husbandry and big fish losses (something we didn't see on any of the farms we visited). This is, in no doubt, down to the research conducted by laboratories such as the TAL and aquatic vets such as Roy visiting these farms on a regular basis to promote fish health management. There have also been improvements in the survival rates of fish during shipping because fish are being shipped in a healthier state. Unfortunately, there still seems some way to go before the shops that then sell these fish onto the public are regulated properly. There are still too many operating with little or no training in keeping fish and promoting good fish welfare. In some aquatic retailers, there is still most definitely an element of playing the 'numbers game', whereby it appears that it is cheaper for them to let certain fish die than keep feeding them until they are sold. I for one feel that stronger regulation is needed for this part of the industry if we are to reduce fish losses through neglect. Perhaps these retailers should be inspected on a regular basis similar to that which happens in scientific institutions.

Following our tour Roy kindly put me in contact with Dr Ilze Berzins (an aquatic vet at Florida Aquarium) to get Amy and I a free pass into the aquarium. It worked and, not only that, we were immediately invited to an evening function at the aquarium to a talk by yet another aquatic vet and friend of theirs, only this time he was an aquatic vet turned astronaut and was speaking about his latest space travels to the Hubble Space Station, amazing! A great evening was then finished off with an invite to dinner surrounded by my new fishy friends, most of them aquatic vets! I found it really interesting just how many aquatic vets there were here in The States and in complete contrast to the situation in the UK, where it is incredibly difficult to find any general vets with aquatic animal expertise. Such a plethora of aquatic vets is obviously a real benefit not just to the pet fish industry, but also to public aquaria and scientific fish laboratories alike and the number of aquatic vets showed me that fish welfare really was of upmost importance here. It was time to say farewell to Roy who had practically given up two days of his time to spend with us and who had been both full of useful information and a great laugh, although often the laughter was at my expense!

With one last day in Florida before the longest part of our travels (3 flights in one day to reach Oregon in the North West - boy this country is big!), we had to take up Eric's second wildlife spotting advice. So, early the next morning, we set off to Crystal River and 3 Sisters Springs – home to a manatee reserve. It quickly became apparent that the unseasonal cold snap



that Florida was experiencing, with air temperatures only just in the 60's Fahrenheit, wasn't such a bad thing this particular day. These low temperatures meant that the warm freshwater springs that stay in the 70's Fahrenheit, except in the coldest of years, were awash with manatees that had come in to escape the cold. We had hired a Jon boat for half a day but with so many manatees about as well as thousands of fish from both marine and freshwater environments intermixing with each other and with a clarity of water I could only liken to some of the best coral reef sites of the world, the time just flew by and suddenly we had been gone for nearly 7 hours rather than our initial 4! We were more than happy, however, to pay the extra half day for the boat hire such was the exhilarating experience we had had and one that will live long in our memories. Thanks Eric! At least we knew being tired the next day wouldn't matter as our three flights to Oregon would allow us plenty of sleeping opportunities.....

### **5) Oregon 23<sup>rd</sup>-25<sup>th</sup> November**

Wow, we made it! The longest day of travelling in our lives and yet we were still in the same country, amazing!! Not much time to recover from jet lag though, as we had a busy schedule, I had two more talks to give about my fellowship in two days and lots of people to meet. First off was a visit to the Zebrafish International Resource Centre (ZIRC), housed on the University of Oregon campus, Eugene, and a meeting with its manager April Freeman (oh and her tiny dog, aptly named Bijou!). This was an amazing facility, and one that most zebrafish researchers will have heard about, because it acts as a repository for wild-type and mutant strains of zebrafish which the scientific community can access. Incredibly, April told me they had just passed the 1000 mark for the number of different strains of zebrafish they held at their facility (each one developed for a specific research application). Interestingly, because ZIRC is an independently funded facility and a non-profit organisation, they actually provide zebrafish strains almost free of charge (only charging the costs they incur) to research laboratories worldwide.

April told me that most of their shipments are of zebrafish embryos as opposed to adult fish, and that this was for a number of reasons including practical, cost saving but, most importantly to take into account, fish welfare. For instance, embryos are much more resilient to being shipped than adult fish and so this immediately reduces stress to adult fish and the risk of losing valuable strains in cargo. Shipping embryos also means less chance of transferring disease to other facilities and, in general, healthy embryos will acclimate better to their new aquarium surroundings/facility than adults. For an organisation that only wants to charge the costs they incur to the user, this also makes sense as it is much cheaper to ship a few hundred millilitres of water containing a few hundred embryos than several litres of water containing the same number of adult fish.

April told me that, in order to ensure the survival of the different zebrafish strains, particularly those that may have taken months or years of research, and at considerable cost, to develop that they cryopreserve (freeze) the sperm from adult males for long-term storage. This also allows them to carry out in vitro fertilisation on embryos to generate a new stock of fish if they

so wish and actually means that they don't necessarily have to always hold the adult fish if they are not required for long periods of time. Amazingly, they even have a travelling team that can go to any laboratory worldwide and freeze zebrafish sperm on site from any new/novel zebrafish strains that they feel are important to make available to other researchers worldwide. ZIRC then ship the sperm samples back to their facility for long-term storage and/or to carry out in vitro fertilisation. April pointed out that this is the fastest and easiest way to proceed. It means that, like when they themselves ship zebrafish embryos to research laboratories, they also never have to deal with adult fish and the associated problems that brings, such as the higher risk of bringing in disease from other laboratories, the difficulty sometimes encountered in acclimating fish to new conditions (e.g. a change in tank size and water conditions) and, importantly and sometimes frustratingly, trying to get the adult fish to breed.

The aquarium facilities at ZIRC almost left me speechless because of the sheer scale of them. At the time of my visit, they had approximately 2500 tanks in operation, which was scary enough, but actually had the capacity for 4000 tanks. This required a near military operation to run and, unsurprisingly, they had a large team of approximately 16 staff and 10 students at any one time to feed and clean the fish. They had three different tank sizes – 20 gallon, 5 gallon and 1 gallon tanks, with the latter size being by far the most abundant. April explained that the large tanks were used to keep lots of animals until the individuals carrying the mutation had been identified and then they were transferred into the smaller tanks and the tanks barcoded for identification. To generate the embryos they needed for their studies, they also carried out the traditional pair-wise breeding methods discussed previously. April told me that they, like the other laboratories I had already visited, had also had some problems with this method when some fish were overly aggressive and even suffered fish losses. They were now adding strips of netting to the small static tanks to act as both stimulus for breeding but also to allow the fish to escape from one another if either one was being harassed.

Despite the fact that the fish all looked healthy and that April was clearly doing an amazing job managing such a large facility, I couldn't help but feel a little sad (again) to see so many small and bare tanks housing zebrafish. My sadness wasn't necessarily just the fact that the tanks were small and bare like all of the zebrafish facilities I had now visited in The States but, more the realisation that even trying to add enrichment to them would be almost impossible. April told me that their focus in terms of fish welfare, like the previous laboratories I had visited, was on providing optimal water quality for their fish and to prevent any disease outbreak. Indeed, their 'plant' facility was the biggest and most impressive I had yet seen. She also pointed out that, with their resident aquatic vet (Katy Murray), they had in fact been carrying out some tests which suggested that maybe the small tanks shouldn't be discounted completely from a fish welfare perspective. Katy and April told me about the two diseases that are commonly found in most zebrafish laboratories – microsporidiosis (a fungal disease) and mycobacteriosis (a bacterial disease) which, if not contained through good husbandry practice, can cause significant health problems to the fish, reducing their ability to

breed (crucial as most research is conducted on embryos) and potentially resulting in moribund fish and/or fish deaths.

They had been conducting some tests investigating disease prevalence in fish housed in their small tanks versus fish housed in their large tanks and, interestingly, found that the fish in the large tanks had a higher prevalence of disease. Both sized tanks were fed with the same water supplies and had similar stocking densities and feeding regimes. So, the only difference between them and possible cause of this result was how the tanks were cleaned. Fish housed in the small tanks were routinely (maybe every 1-2 months) captured and transferred into a clean tank. The tank itself was then emptied, removed from the racking system, and sterilised by washing at a high temperature before being replaced to the racking system ready for the next consignment of fish. This wasn't a practical solution for the fish housed in the large tanks because the tanks were too big for sterilisation, and anyway there weren't enough spare large tanks to transfer fish from one to the other. Instead, these tanks were cleaned in-situ, simply by wiping the glass and siphoning out the waste once it had settled to the bottom of the tank, all the time with the fish still in the tanks. They found that the fish would feed on this waste (made up of algae and bio-films that naturally grow on the glass) as it was being cleaned, and that this was the likely source of the disease. If this process was repeated enough then the fish were more prone to showing health effects caused by these diseases (and others) harboured within the algae and bio-films. They also felt that cleaning the large tanks with the fish in-situ could be stressful in itself to the fish. Therefore, any undue stress the fish suffer during this process will also make them more susceptible to infection. Interestingly, this is something we have found in our laboratory at Exeter and, despite our large tanks, we still rotate the fish every month or so into clean tanks. Our tanks are also far too big to be removed and sterilised and so we adopted a strategy of emptying the tanks, cleaning them thoroughly with ethanol (to sterilise) and then leaving them overnight to allow any residual ethanol to evaporate off before filling them and adding a new set of fish. We tend to keep one or two tanks available on each rack to allow us to carry out such a rotation and this dramatically reduces any instance of health problems in our fish. Often the change also stimulates the fish into an even greater breeding frenzy and I think this process even prolongs their egg laying duration.

Following our tour of the facilities at ZIRC, I then gave my talk for the third time. April then kindly took us just across the road from her facility to meet Tim Mason, facility manager for the Zebrafish Facility at the University of Oregon, Institute of Neuroscience. This facility was home to world renowned scientist Professor Chuck Kimmel who had worked alongside Professor George Streisinger (famed for pioneering the use of zebrafish in scientific research). April gave us a quick tour of Tim's facility which was another very large aquarium with numerous small tanks racked almost from floor to ceiling that housed over 80,000 zebrafish and that were used to support 14 research laboratories and the biology curriculum at the University. I then went to meet Tim for a brief chat to see what his views were on enrichment for zebrafish and fish in general.



Interestingly, Tim was quite forthright and didn't agree with the need for enrichment at all, stating that zebrafish are such a hardy species that they will readily breed in bare tanks and that their hardiness is why they were chosen for research purposes in the first place. He also pointed out that their regulators were not asking for research into the use of enrichment to improve fish welfare. Additionally, he told me that their own vets require all tanks to be kept sterile and clean and that this was to be standardised throughout all zebrafish facilities so that the fish could be observed quickly to inspect their health. With so many tanks to look at, anything obscuring the view of the fish would just delay such checks. Tim said his only focus from a welfare perspective was on providing good water quality for his fish but that, in addition, he always used zebrafish systems that provided an air supply to each of his tanks such that, if the water stopped for any length of time, the fish wouldn't suffer before the water supply was fixed. This air could, he felt, be considered a type of basic enrichment for the fish as zebrafish can often be seen swimming amongst the air flow. Tim finally went on to say that he thinks the most important thing for the welfare of zebrafish is to separate people caring for fish from people doing research and that he didn't agree at all with the sentiments of the official zebrafish laboratory book which suggests that if you are not sure whether a fish is happy or not then put yourself in the fish's perspective and see if you would be happy – he described this notion as 'rubbish'. Although I found myself not agreeing with many of Tim's sentiments, as he was at completely the opposite end of the spectrum to my views on the need for enrichment, it was certainly interesting to get his 'take' on the subject. Following our discussions, it was time once again to head off and get ready for our next visit and location of my fourth and final talk of the trip at Oregon State University.

Amy and I arrived in Corvallis early the next morning and waited at a pre-arranged meeting place for my next fishy friend, Jan Spitsbergen (research assistant professor at Oregon State University) who runs the Centre for Fish Disease Research at the John Fryer Salmon Laboratory. She had recommended that if we arrived early we should wait for her in one of her favourite bakeries and sample something for breakfast until she arrived. Oregon had already seemed quite different to the rest of America we had visited so far with almost a more tranquil feel to the air, less hustle and bustle and certainly a change to more healthy offerings when it came to food. Everything seemed fresher and there were lots of nice local food shops more akin to a small town/village, and I for one couldn't resist the offerings of the bakery Jan had recommended! Jan arrived and, following another cup of tea (the nearest we'd had to good old English Tea since we arrived), she took us back to her laboratory to give me an insight into the work she was involved in and that of her colleagues at Oregon State University, where I would give my fourth and final talk.

The John Fryer Salmon Laboratory where Jan works has been at the forefront of fish disease research for over 30 years and has identified the causes of several important diseases afflicting both wild freshwater fishes, in particular salmon stocks, but also wild marine fishes, ornamental species, and fish used as research models, including zebrafish. They have developed many diagnostic tests such that these diseases can be identified and have

produced vaccines to help protect against some of them. Jan herself is a fisheries scientist and veterinary pathologist who has worked in fish pathology for over 20 years and is currently conducting research into carcinogenesis (formation of cancer cells) using the zebrafish model. She explained that she was investigating the relative roles that diet, husbandry systems (why, therefore, she was interested in my visit on fish welfare), infectious agents and genetic influences play in this process using various strains of zebrafish. Interestingly, Jan then told me (and gave me a huge folder with the full course notes) about a course that she and her colleagues ran called 'health management of laboratory fish' (covering everything from water quality to identification of fish diseases), including for zebrafish. This was something that I immediately felt I should enroll on in the near future.

Jan then took us on a tour of their aquarium facilities which were very large and, although mainly set up for work on salmon, had a small area for zebrafish. The zebrafish were in large tanks, more akin to my holding tanks here at Exeter and, although bare, did have some algal growth. Jan thought this growth was good for reducing the reflective quality of the glass, making the fish more settled, particularly those prone to nervousness such as some strains of wildtype zebrafish. However, through her research, she had found that this algae contained toxins and carcinogens and was also linked with an increased incidence of microsporidia infection when compared to tanks that can be sterilised. This was similar to the findings of April and Katy at the laboratory in ZIRC and something Eric Herbst had alluded to when discussing the benefits of his Aquatic Habitats system. Therefore, Jan surmised that clean tanks might be better for reducing disease prevalence but at the same time recognised that they may not be so good for reducing stress caused by the reflective qualities of glass. She suggested that dark bottomed tanks could be used instead to reduce long-term stress, and as a consequence would potentially increase egg production. Although it is more difficult to see when dark bottomed tanks are dirty (we have some at Exeter), Jan suggested that this was easy to overcome with a standardised maintenance schedule. Interestingly, Jan also mentioned how flow-through systems (where water is fed into the tanks and then goes straight to waste) are much better at reducing disease prevalence than re-circulating systems (where water goes to the tanks, then to a filtration unit and then back to tanks) which are more commonly employed in research laboratories to conserve water and save on costs. Interestingly, Dr Mike Kent (Microbiology Department at Oregon State University and someone Jan would introduce me to later that day and whose research interests were largely focused on investigating diseases of importance to zebrafish) has observed that zebrafish raised from eggs in a flow-through husbandry system did not develop microsporidiosis but did develop it when raised in re-circulating systems. Microsporidiosis is such a universal problem in zebrafish facilities that Mike Kent's research group has now gone as far as developing diagnostic tests to screen zebrafish broodstock in order to derive specific pathogen-free strains.

Before heading to the main campus to meet Mike and colleagues, Jan took us to meet Robert Tanguay, associate professor and director of the National Institute of Environmental Health Sciences (NIEHS). The mission of Robert's laboratory and that of NIEHS is (through the use of the zebrafish

model) to try to reduce the burden of human illness and disability by understanding how the environment influences the development and progression of human disease. Robert showed us his zebrafish facility which was equipped with racks of small to moderate sized bare plastic tanks (similar to the other facilities we had visited) and had capacity to rear up to 10,000 experimental zebrafish in addition to holding brood stock to support his research. Fish welfare in his aquarium was, again, focused on providing optimal water quality (his aquarium had large scale modern filtration equipment) and disease prevention, as opposed to enrichment. He said that he screens any new batches of embryos for microsporidia by sacrificing half of them for diagnostic tests (developed by Mike Kent's laboratory) as well as the parents and, if they are 'clean', the remaining half of embryos are kept and raised for his next generation of fish. Robert said that he was also keen, and felt it important for the welfare of his stock, to use large numbers of fish in mass breeding groups, rather than pair-wise breeding to generate his new stock to prevent inbred traits developing and potentially fish of poorer health that may also be more susceptible to disease.

Following my discussions with Robert at the John Fryer Salmon Laboratory, Jan then took Amy and me to meet with Dr Mike Kent and Professor Carl Schreck. Carl is a Professor of Fisheries and Wildlife, whose research focus is directed at understanding how the environment and genetics control physiological performance in fish, notably stress and reproduction. What was most interesting for me was that Carl and Mike had just been supervising a PhD student (Jennifer Ramsay) who, during her PhD, had been investigating the role of husbandry stress on physiological performance in zebrafish. Although Jennifer wasn't working on environmental enrichment for zebrafish, she was working on two other important facets of zebrafish welfare.

The first of these was crowding stress in zebrafish, which can also be applied to other cultured fish used in laboratories, as well as fish raised for the pet industry, fishing industry and for the food market. As we had seen zebrafish are often confined to small tanks and/or over-crowded during their life-time. Secondly, Jennifer was also working on handling stress in zebrafish (again equally applicable to other fish in the aforementioned industries) which are frequently handled during husbandry and experimental procedures in the laboratory (for example laboratories using the traditional pair-wise breeding method as previously described). Although both crowding and handling stress commonly occur, Jennifer told me we still know little about the health effects these stressors have on the fish. Stress is often implicated in laboratories when breeding is poor and during outbreaks of disease. Therefore, understanding how zebrafish respond to such stressors will allow improvements to fish handling, health and fitness whilst minimizing husbandry-associated sources of variation in experiments. Interestingly, it is this last point that Jennifer raised - improving the consistency and reproducibility of studies, rather than improving fish welfare per se that is more likely to grab the attention of the scientists. So, if studies such as Jennifer's can show researchers that the quality of their science can be improved by understanding more about the conditions in which these fish should be kept and the husbandry practices that should be adhered to, then this is more likely to create improved welfare for this species and hopefully put to bed the notion



that they are so hardy that they can 'perform' under any conditions. It was again interesting to realise the potential of the zebrafish model for research, as any findings on the effects of husbandry stress could be extrapolated to stress in other laboratory and aquaculture raised species.

I had learnt a lot from my time in Oregon as it turned out to be a real 'hotbed' of world class research, particularly into diseases and stress responses of fish, but it was time yet again to move to pastures new and the last part of our trip - to California and to arguably the most well known aquarium of them all, Monterey Bay, I couldn't wait....

### **6) California 26<sup>th</sup> November – 2<sup>nd</sup> December**

After a long overnight bus journey, on the famous Greyhound buses of America, we arrived in Sacramento, California. Amy had booked an economy car to cover the long distances we would be travelling in the vastness of this State only to be told on arriving at the hire car company that they only had one car left in the yard which we would have to take. Incredibly, and we thought they were joking at first, it turned out to be a 4L convertible Mustang – not quite the economy car we were expecting!! Anyhow, we had little choice but to take it and, as it turned out, a pretty good deal as they gave it to us for the same price as the economy car would have cost – about \$20 per day!! So, after squeezing in our suitcases, we were on our way – first stop Yosemite National Park, staying in a hotel in Oakhurst, near the park boundary. We spent a couple of days exploring the park being wowed. Firstly, by the giant sequoia trees – some nearly 3000 years old and so tall and straight as they pierced the skyline like skyscrapers in a big city. Secondly, by Yosemite Valley - the mountain views at Wawona tunnel and various waterfalls along the way. Turns out the mustang wasn't ideal for such terrain but that was about to change as we headed for our final destination, Monterey Bay Aquarium, where I was lucky enough to be meeting up with one of its curators Steve Vogel.

The Monterey Bay Aquarium (MBA) was founded in 1984 and is located in one of the most picturesque settings, right on the Pacific Ocean shoreline in Monterey, California, and within the Monterey Bay National Marine Sanctuary (MBNMS), a Federally-protected marine area. It is this setting and its close proximity to the sea that Steve told me allowed it to have exhibits quite different to many other public aquaria, because it allowed them to pump seawater directly in from the Bay. Steve told me that this design made some of their exhibits ecologically essentially part of the ocean in the bay and allowed the culture of organisms not achieved in ordinary enclosed seawater systems. During the day, the seawater is filtered to maintain clarity so that the public could view the tanks inhabitants unhindered. However, at night, the seawater is unfiltered and, therefore, importantly and uniquely for this aquarium brings in with it natural food items such as plankton for some of the exhibit's residents (including some of the fish species) to feed on. Steve told me that this design also allowed Monterey to be the first aquarium in the world to grow Californian giant kelp, a type of seaweed that can grow as fast as half a metre a day and ultimately reach tens of metres in length. This kelp forms the centerpiece of its most iconic exhibit representing local coastal

marine life and can only flourish here because, as well as food for the animal life, the unfiltered seawater also brings in with it the essential nutrients, salts and trace elements that the kelp needs to flourish. In addition, the aquarium was also fitted with a wave machine to provide the water movement and turbidity kelp needs to grow and the exhibit was almost exclusively lit by natural sunlight. Steve felt that it was this combination of all of these factors that really allowed them to maintain such a diversity of life in a single exhibit and that he felt was just not possible to recreate in an enclosed and/or artificial system.

Interestingly, and as if this exhibit wasn't natural enough, Steve also said they introduced a shoal of live sardines into the tank every couple of months as a food source for some of the larger predatory fish to really complete the eco-system effect. They were careful not to introduce any parasites when they introduced the sardines by quarantining them first. Steve said that, as the water from the exhibit went straight back into the Bay (a marine sanctuary), they had to be especially careful not to introduce any disease and certainly couldn't use any chemical treatments if any of its inhabitants became ill. Steve felt that the introduction of the sardines, as well as new species periodically to the exhibit, also provided a certain level of enrichment to the inhabitants of the tank as it meant there was a constantly changing dynamic to the exhibit, something that would happen in nature. The use of live food itself may be somewhat contentious but, in order to keep some fish species, this is essential as certain species won't feed on anything other than live food. The use of live foods as enrichment is an area that raises differing views from people, with some suggestions that these species shouldn't be kept in captivity at all for this reason alone, however, this is really beyond the scope of my discussions here.

Steve then kindly took Amy and I behind the scenes of some of their exhibits including, excitingly, their largest exhibit representing the open ocean. This contained some huge pelagic fish species (for example, tuna, dorado and sharks) some of which represent the fastest swimming fish species in the world. Steve pointed out how they had been working to ensure that this tank was shaped as smooth and rounded as possible to prevent these large fish from damaging themselves as they patrolled around the tank at great speeds. The tank had also been tiled with blue tiles and this, combined with the correct lighting, gave the tank the blue open ocean effect that these fish would experience in the wild. Steve told me about the difficulties of housing some of these fish satisfactorily when they become full size. In fact, he told me they have a policy to release fish back into the wild if they had outgrown their exhibit or could no longer be kept to a high enough standard. Interestingly, Monterey is one of only a few aquariums that has successfully kept juvenile Great White Sharks (in this open ocean exhibit) for any length of time and that has successfully released them back into the wild once they had out-grown the exhibit. Steve told me the unique design of the Outer Bay exhibit (with its feed of natural seawater from the Bay) had played a central role in keeping the juvenile white sharks healthy during their time in the exhibit. He also felt that, potentially, this explained why Monterey were able to maintain Great White Sharks for so much longer than any other aquariums had managed in the past. By working in close conjunction with its sister organisation, 'The

Monterey Bay Aquarium Research Institution' (MBARI), renowned worldwide for its research on deep-sea marine life and other programs in marine biology, the aquarium has now, in fact, successfully hosted juvenile white sharks on several occasions, and the staff have developed a method for their careful capture, housing and transportation, and release. The procedure of tagging these animals is also enabling the research teams at MBA and MBARI to understand more about the natural biology of this much maligned species which can only help with conservation efforts and the provision of information to the public through future captive programs.

To finish off the tour, Steve showed us to their only mammal exhibit in the aquarium which held marine otters, captured from the Bay, some as orphaned babies. Otters are known for their inquisitive and intelligent nature and Steve said that this was one exhibit that really required them to think about new and novel ways to keep them stimulated as they quickly became bored, or too quickly solved the problems put in front of them. Steve said the type of enrichment they provided often centered on their food. Firstly, they provided them with a wide variety of food items that required them to use different skills in order to tackle eating them and this often included live food (e.g. shellfish) from the Bay. They also hid their food to encourage foraging behaviour and to test out their senses and they even put food in ice blocks. The otters were also provided with lots of toys of various forms as they are very playful animals, having often been observed in the wild playing with objects they find. The key, Steve said, was to provide as much variety as possible for them and keep repetition to a minimum.

Steve went on to tell me that they usually appoint one or more members of staff to each exhibit and/or species, and these staff were then responsible for researching the husbandry requirements (including enrichment needs) for that particular exhibit/species. This meant that there was a continuous improvement program operating for the welfare of the animals in their care. Steve also said that many of the staff that worked for them were well qualified with Bachelor of Science Degrees in relevant subjects and that they were encouraged to go on training courses in fish husbandry as well as to visit other aquariums and research establishments that already have information on a particular species they may want to keep. In fact, one of the richest resources for them was the communication they had with other aquariums and the freedom to share that information. Interestingly, Steve told me that the aquarium also had its own resident aquatic vet as well as a local vet on standby so they were quickly able to deal with or identify potential health problems for their animals well before they became too serious, and important to maintaining the high welfare standards they set themselves for the animals under their care.

Following my discussion with Steve, we then fortuitously bumped into one of the staff looking after the freshwater exhibits, John Lambert, who was just about to feed some of his fish at the time we met. It was good to bump into John as it turned out he, like me, was also a keen fish hobbyist and it was clear that he brought this knowledge with him to the exhibits under his care. John talked me through a number of his displays which he said were, in the main, set up to represent/mimic certain freshwater bodies of the world with the correct species and water conditions – so called biotype correct aquariums.



One exhibit that particularly caught my eye was a very nice African lake set up, with the appropriate fish, plants and invertebrate species from that area all living in harmony together. John suggested that these types of tanks bring out the best in the species in terms of their natural behaviours and that they are also far more inclined to breed – a sure sign that they are healthy and thriving. Also, unlike tanks which just have a mixture of fish (and other species) from different parts of the world 'thrown' together with little thought other than immediate, but short term, impact to the public, these exhibits actually looked and felt like mini-ecosystems in action. All the species were interacting with each other as they very much would in the wild. This was certainly the case with a number of John's tanks. John was just feeding his exhibits live food, although not other fish as in the large marine tanks I had seen earlier with Steve, but small plankton and worms similar to what the fish would naturally feed on in the wild. He felt that this made a significant difference to the fishes' behaviour as it encouraged them to forage for their food (many of these tanks were heavily planted, or had lots of wood and rocks) and that it brought out the best condition (including colour intensity and breeding displays) in the fish, significantly more so than dry food alone.

I went on to ask John if, like me, he felt it a shame that most aquariums afford huge dynamic displays for their marine exhibits and yet the freshwater displays are often restricted to small tanks in comparison. Maybe this is just a result of me being more of a freshwater fish enthusiast but, aside from a few tanks I have seen (particularly at Tennessee Aquarium), almost all public aquaria are weighted heavily in favour of their marine displays and I for one feel the public are losing out on understanding the rich diversity of our freshwater habitats. John could see my point and went on to tell me that the amazing exhibits he had set up and that were now looking at their very best after 2-3 years of maturing were about to be dismantled for new exhibits! Such is the demand for continual change to keep the paying public enthused and coming back time and again – such drastic changes rarely happens to the larger marine displays I thought to myself!!

Once again, I had found that I had been chatting so much about fish that I had completely lost track of time and only when it dawned on Amy and I how very quiet it suddenly had become in the aquarium, did John point out that it had in fact shut some time ago. Jon had kindly sacrificed some of his own time for us – not that he seemed to mind as once two fish enthusiasts get together they can talk about fish almost indefinitely. John unlocked the entrance door and we said our farewells. Having wanted to visit this iconic aquarium for most of my life, it was a definitely a shame to leave.

Amy and I had one day left in California before we headed back home to the UK after what had been 4 amazing weeks in America. We didn't want to waste our last day and so we headed off for one last hurrah in our 4L Mustang down the Pacific Coast Highway, firstly taking in 17 mile drive in Monterey Bay and Pebble Beach (home of one of the most famous and picturesque golf courses in the world and, as it turns out, the tastiest panini - steak and shrimp - I have ever eaten, even if they were \$10 a pop) before making it, just as the sun was setting, to see a large elephant seal colony at Hearst Castle. This was a great way to end such a memorable trip and, having never been to

America before this visit, we knew we would have to come back very soon. There was just too much to do and see not to return. Thankyou Winston!

### **7) Summary of findings in America**

- 1) Laboratories in America, unlike in the UK, are not being asked to provide environmental enrichment
- 2) Vets and regulators want bare tanks so that they can quickly and easily assess fish health
- 3) Zebrafish are typically maintained in commercial racking systems with small tanks because of the need to hold lots of different zebrafish strains for medical research
- 4) Commercial racking systems with small tanks, albeit excellent for maximising tanks numbers in a small space, are not conducive to enrichment
- 5) Focus of fish welfare in laboratories is on providing optimal water quality using high specification filtration equipment
- 6) The addition of enrichment to pair-wise breeding tanks (addition of plastic plant and sloping base to mimic shallow water observed in the wild) has, however, increased egg production and decreased fish deaths
- 7) Despite (6), there is still a belief that there is insufficient evidence that environmental enrichment is beneficial, particularly if fish have only ever experienced bare tanks
- 8) Although currently very few studies on environmental enrichment, other aspects of fish welfare are being investigated such as stress caused by crowding and handling pressures – a positive step forward for improving zebrafish husbandry
- 9) Some novel techniques to improve zebrafish husbandry are being developed by industry e.g. MEPS system courtesy of Aquatic Habitats reduces handling stress whilst maximising egg production for research
- 10) Compromise probably needed between welfare and research needs – no one expecting laboratories to adopt fully biotype-style aquariums as seen in public aquaria – even small changes can be beneficial to start with, such as reducing reflective surfaces
- 11) Environmental enrichment strongly used in public aquaria – knowledge can help guide what might work and/or be worth trying in laboratory settings
- 12) Lots of expertise in America on fish diseases, some researchers even specialising on zebrafish diseases, and a significantly higher number of aquatic vets available than in the UK – more expertise can only be beneficial for the fish under their care
- 13) Very good communication between fish research laboratories and such shared experiences are a powerful resource for improving fish husbandry practices
- 14) The Zebrafish Husbandry Association (ZHA) was very vibrant – similar zebrafish group established here in the UK but as yet not as active on issues of fish husbandry

- 15) The healthy debates going on between the researchers' needs and fish welfare, and the fact that the success of both is clearly interlinked, can only be good for the future welfare of laboratory maintained fish

***Phase 2 of my fellowship; 4-weeks in Bangladesh (27/04/09 – 25/05/09)***

It seemed like no sooner was I back from my amazing experience in America than it was already time for me to leave on the second phase of my Winston Churchill Fellowship. Could I have chosen a more contrasting destination? Probably not, but that was not up to me, as my next destination was chosen for me on the basis that it was the native home of the zebrafish. This was the very fish I had seen in their tens of thousands in bare tanks in laboratories across The States, and that I kept back at Exeter, and I was finally off to see how they lived in their natural habitat, the freshwaters of Bangladesh. I was lucky that on this trip I was able to have another travelling companion in the form of PhD student, Charles Hazelrigg (jointly supervised between the University of Exeter and Imperial College London), who was also researching zebrafish, albeit not for welfare, for his PhD. We decided to arrange our trips to coincide with each other such that we could help each other with our research as we would both be searching in the same region for the same fish species. Fortunately, we were both avid sports fans and, importantly in this part of the world, keen cricketers – something that would come in useful during our stay in what we discovered to be the most welcoming of countries.

We set off on our journey leaving Heathrow airport on the 27<sup>th</sup> April with our bags packed full of all sorts of equipment that we would need for catching zebrafish and measuring various water parameters, books for identifying fish and lots of light-weight clothing to cope with the heat and humidity and not least a small amount of trepidation that I certainly hadn't felt when heading to The States. Eighteen or so hours later, following a brief stopover in Delhi and the 5 hour time difference, we safely arrived in Bangladesh's capital city, Dhaka, after what were two excellent and comfortable flights with Jet Airways. We collected our luggage and headed out of the airport at which point we were immediately hit by the intense heat and humidity of this country. I think it was in the high 90's Fahrenheit and about 80% humidity when we arrived, not ideal for a pasty Englishman (Charlie was pretty brown - students!) that hadn't seen the sun for a while, but was I to blame for this, or was it the doing of our shocking summers in the UK over the last few years? Anyway, we checked into our hotel in Dhaka where we would spend one night before heading to our final destination, the University's own International Guest House directly opposite the Bangladesh Agricultural University (BAU) just a few kilometres south of the City of Mymensingh. Following a brief walk around Dhaka before nightfall and our first experience of the hustle and bustle of this country, particularly the traffic, oh! and our first experience of a tuk tuk (a small 3-wheeled motorcycle with a cabin that covers the driver and passengers), we were in bed fairly early. This was mainly if I'm honest, not to catch up with the lack of sleep from our long but comfortable journey, but more so that we could get up at 2am and watch the Champions league football live – Chelsea versus Barcelona, semi-final first leg!



The following morning, we were picked up early (both wondering whether we had been wise to watch the football!) in a nice air-conditioned car and headed out of Dhaka and for 80 mile or so journey to the BAU and our guest house. Boy did this journey open our eyes to travelling by road in this country. The 80 miles would take us over 3 hours on a somewhat bumpy road and the few times we managed to briefly fall asleep when there was a smooth stretch, we would quickly wake to the sound of horns beeping (used almost continuously by drivers in this country) only to see a huge lorry or bus heading straight at us. I don't know what we were worrying about though.....a quick swerve by our driver or the driver of the oncoming vehicle, sometimes briefly leaving the road, and we were able to keep going on our merry way with no interruptions and certainly little need to use the brakes!

Thankfully, we arrived unscathed at the guest house where we would be staying for the next 4 weeks and we were somewhat taken aback as it wasn't quite the lifestyle we were expecting in Bangladesh. Although our accommodation was basic, it was spacious, and we each had our own room complete with double bed, en-suite bathroom and, most importantly, a good powerful shower with plenty of hot water. To add to our hardship, we had a lounge with TV that amazingly showed more sport than is available to us back in England (including choice of 3 live premiership football matches on a Saturday!), our own dining area, a large veranda with views across a (our!) well manicured garden (complete with a supply of bananas, coconuts, lychees and jackfruits) and, to top it all, the garden backed straight onto the old Brahmaputra river giving us excellent views – amazing. Oh, did I mention we also had our very own resident chef (Mukul) who would be cooking us three cooked meals a day and his assistant (Riaz) who did all of our laundry (including ironing), and fetched us endless cups of tea (good tea as well, but I suppose that was expected as we were in a tea growing country). Needless to say, we settled in very quickly and any thoughts of trepidation about travelling to Bangladesh were soon banished. In fact, as we settled down to play a bit of dominoes whilst taking in views from our veranda, it was going to be hard to leave the comfort of our accommodation to explore Bangladesh, but search for the elusive zebrafish we must.

The next day, we headed across the road (literally 2 minutes walk) from our guesthouse and arrived at the BAU. Here, we met up with Professor Wahab, (Professor of Limnology and Aquaculture in the Department of Fisheries Management) and with whom we had organised our respective trips from the UK and who would be our main point of contact for the duration of our stay here. Professor Wahab was extremely welcoming and immediately started chatting to us about how the BAU had been established in 1961 with the aim of educating its people in all aspects of agriculture such that it can produce first-rate agriculturists, agricultural scientists and researchers for shouldering the responsibilities of agricultural development of the country. It hadn't taken us long on the trip from Dhaka to our guesthouse to realise just how important farming is to this country. It seemed like every square inch of land was being farmed either for rice (the staple food) or for fish (the main source of protein) and that for many Bangladesh people their entire days were consumed with food production. Interestingly the land was much greener and fresher than I had imagined, despite our visit coming at the end of their dry

season. Cows and goats had dotted the landscape, tethered to trees or stakes, everywhere you looked, and funnily enough even including within the campus grounds – no good land was spared.

Professor Wahab told us that the BAU campus was large (in fact it is spread over an area of 4.85km<sup>2</sup>) and had a number of specialist faculties including Agriculture, Animal Husbandry, Fisheries and Veterinary Science. He took us to where we would be based within the Faculty of Fisheries (in the water quality laboratory) which conducted undergraduate and postgraduate degree programmes in fisheries biology and genetics, aquaculture, fisheries management and fisheries technology. Professor Wahab told us that this area (Mymensingh) was the major production area for all freshwater fish and prawn aquaculture in Bangladesh and that BAU was critical in supporting this industry by producing educated and skilled workers that could keep pace with changes in modern technologies in farming through studying its degree programs.

Charlie and I were then introduced to a number of Professor Wahab's post-graduate students, including Rahman (PhD student), Ripon (MSc student) and Hira (MSc student) who would all turn out be extremely kind and helpful in supporting us during our stay. We were also introduced to Professor Mostafa Ali Reza Hossain (Department of Fisheries Biology and Genetics), and a number of his post-graduate students, in particular Nahid (PhD student), that worked in the laboratory next door. Interestingly, at the time of our visit, Professor Hossain and Nahid were trying to collect as many (ideally all) of the different fish species native to Bangladesh for a fish museum and biodiversity center that they were just having built at BAU. Fish are so important for food to the people of Bangladesh, yet few realise the richness of the species that are present all around them (over 260 freshwater fish species) and this museum was being built to showcase, as well as educate, people of the richness of their fish fauna in the hope to protect it. This was great news for a fish enthusiast such as me and I felt lucky that my trip had coincided with such a venture and as it transpired I would end up spending a lot of time with Nahid and colleagues in their laboratory identifying various fish species for their new museum. Lastly, but by no means least, we were introduced to Professor Zoarder Faruque Ahmed (Department of Fisheries Management), who with his experience collecting fish in the field, including zebrafish, had kindly offered to help us find and catch this species. Professor Zoarder was a great character and we would see a lot of him during this trip as he would frequently pop over to our guest house in the evenings, actually to work but ultimately we would all end up playing dominoes and chatting for hours instead.

So now that we had been introduced to everyone and had seen the campus and the laboratory that we would work in, we were ready to start searching for zebrafish. We started our search actually within the BAU campus grounds in their field station. This had an impressive collection of dozens of different sized ponds, anything from approximately 50 square metres up to many hundreds of square metres in size that were being used for various aquaculture trials on fish and prawn production. However, within these very ponds, and especially in some ponds that had remained unused for a while, we were hopeful for our first catch of wild zebrafish and we weren't to

be disappointed. Following several trawls with a large fine mesh seine net across a number of different ponds with no joy, finally we would hear the call of 'ANJU' (the Bengali word for zebrafish, meaning beautiful) coming from our net men. They had finally captured two adult zebrafish from one of the less used ponds in the field station that had become recolonised with lots of plants and as they brought the net close to Charlie and I on the bank we caught our first glimpse of wild zebrafish. My first impression having seen thousands of zebrafish in the laboratory and in pet shops and indeed even in my own home aquaria as a boy was that these wild fish were indeed 'ANJU' with stripes that seemed so much bluer than any I had seen before. Although they weren't in great abundance in this pond (just one or two caught per net), we were both pleased to have seen them so early on in our search and felt reassured that with our Bangladesh team we stood a good chance of finding many more over the coming weeks.

What was also exciting, particularly for me, was the number of other fish species (at least 10 in our first pond) that we caught living alongside the zebrafish, along with strange looking aquatic insects and, in one haul, even a snake! Some of the fish were easy for me to identify as they were fish that were common to the pet fish industry, such as an amazingly beautiful adult dwarf gourami (*Colisa lalia*), spectacular in colour with its vertical red stripes overlaying its almost translucent blue body. What a fish enthusiast would give to have one of this quality in their home aquaria! However, other fish we caught would require identifying back in the laboratory with Professor Hossain and Nahid's expertise.

It suddenly dawned on Charlie and me what a crowd we had drawn even within the confines of the BAU campus all vying for a place next to us on the banks of the ponds to see what we were doing. There certainly seemed to be some puzzled/bemused expressions on their faces clearly wondering why we would be going to so much effort to catch such a small fish? After all, everything here was about food and, although some of the fish the Bangladesh people ate were small, zebrafish certainly weren't on their list. I felt it a shame that we couldn't convey to them how important their little fish was to science, but at the same time couldn't help wonder whether these people would ever benefit from discoveries made off the back of the very fish that swims amongst them as they farm their fish ponds and rice paddy fields.

Over the next few days, Charlie and I would get to know the ponds within BAU's field station really well, coming up either early mornings or late afternoons so as to avoid the intense mid-day heat, when not only was it too hot for us to work but even the fish took cover disappearing amongst the shade of the vegetation. Some days we were even lucky enough to be joined by fish eagles and kingfishers (not ideal for the students using the ponds for experiments!) but it made it a memorable and tranquil place to be. Aside from the wildlife watching, we quickly built up a routine between us such that at each site when we located zebrafish we would keep netting the area until we had a minimum of 20-30 fish (the minimum number we needed for our analyses). Once we had reached these numbers, we would transfer the fish to some large glass tanks at BAU's aquarium facility (near to our laboratory), to be sorted at a later date from the myriad of other fish species we had caught at the same time and that we needed help in identifying. We would then take a





**Figure 5. Collection of zebrafish and associated habitat profile data from their native habitat in Bangladesh. Top row: Our nets men pulling a seine net through weedy ponds. Second row: Our first glimpse of a wild zebrafish and a beautiful dwarf gourami in the same net – fish hobbyists would be jealous. Third row: Me and Charlie collecting plant and water samples. Bottom left: Typical gathering of local onlookers with bemused faces! Bottom right: Measuring our catch of zebrafish in the laboratory – they had amazing blue stripes, almost fluorescent.**

whole series of measurements at each collection site including the size of the pond, water depth, water clarity, temperature, pH (water acidity), conductivity (chemical/salt composition of the water), water samples for later laboratory analyses (e.g. ammonia, nitrite and nitrate levels), percentage of vegetation cover, plant samples for later identification, whether the pond was natural or artificial, annual or perennial in existence, and whether it was being used for anything in particular, such as for aquaculture. Essentially this information would allow me to build a detailed profile of the type of environments in which zebrafish are found in the wild. I then hoped to use this information not only to improve our understanding of the natural biology of the zebrafish but, and the reason why I wanted to come here for my fellowship, to see if this information could be used to improve the welfare of the zebrafish we keep back in our laboratories.

Following the collection of these data, we would then go about the lengthy task of separating the zebrafish from the other fish species and take them to the laboratory so that we could measure the length and weight of every individual zebrafish we had caught. This would provide us with useful information on the size distribution of different populations of zebrafish that we could compare back to our laboratory strains. Unfortunately, and as is often the case in science, we needed to sacrifice a small number of individuals from each population so that we could bring them back to Exeter for further analyses to learn more about their biology, in particular the ratio of males to females and their reproductive status relative to size. Again, this would provide invaluable information on the biology of this species that is so widely used and yet which we still know so little about. All of the remaining fish (including the other species for identification) were carefully kept alive during this process and later released back to their original ponds.

Working in the laboratories at BAU was an eye-opening experience for Charlie and I as we hadn't anticipated the disruptions caused by regular power cuts and that always seemed to happen when we were in the middle of weighing our fish. This almost daily occurrence (sometimes multiple times per day) made us realise the things we took for granted in our laboratories back home and how we would never have problems trying to carry out such simple tasks as weighing fish let alone any of our more onerous analyses that might depend on permanent power for days! Worst still for us was a power cut meant the end of our nicely air-conditioned laboratories and the uncomfortable feeling of working whilst dripping with sweat, not nice at all! However, such episodes certainly added to our experience of living and working in Bangladesh, but more importantly made us realise how impressive it was that the academics and students managed to carry out such good quality research.

Going in search of equipment also led to some interesting experiences into Bangladesh life. For instance, the purchase of a scientific thermometer, some batteries for our portable aerators and some buckets for our fish meant a one hour round trip for me and Rahman to Mymensingh City in a cycle rickshaw (a small 3-wheeled human powered bicycle with a hard seat at the back to carry up to two adult passengers)! I couldn't understand for the life of me why Rahman was having such difficulty trying to get a rickshaw driver to take us on our journey. Only after conversations with at least seven or eight

drivers did we finally get a ride but it was only at the end of our journey (when we paid) that I discovered why – Rahman drove a hard bargain! By the end of our hour long journey, the driver of our rickshaw had gone from wearing a nice, clean baggy shirt to one that on our return was completely soaked through from the exertions of pedalling two grown men over fairly arduous terrain in the mid-day sun and, all to my horror, for the equivalent of 20 pence, astonishing! It was a great way to experience the hustle and bustle of the Mymensingh City though, albeit I wouldn't recommend spending much more than an hour squeezed on a tuk tuk with a hard wooden seat – pins and needles have never felt so painful!

It wouldn't be long before Charlie and I had exhausted our search for zebrafish within the ponds of the field station at BAU (we had managed to find zebrafish in a total of three ponds), and so we needed to start searching further afield. To begin, with we headed off by foot with our friends Rippon and Hira to guide us and help us carry our sampling equipment. Within metres of leaving the field station, we were walking between fields of rice that stretched as far as the eye could see. Interestingly, these fields were all interconnected in some way by various drainage ditches or natural water channels that were chock-a-block with plants. We stopped at various points along our walk, particularly where there was a slight break in the vegetation and cast (dragged) our seine net through the narrow ditches (less than half a metre wide) until eventually, yes, we struck gold and had found more zebrafish. We continued with this method on a couple of days and managed to find enough fish from two new locations in drainage ditches whereupon we took all of our measurements to build up a detailed profile of these habitats.

By this point in our trip, Charlie and I had already experienced one or two heavy rain storms and had witnessed firsthand how quickly the low-lying land here within the Brahmaputra catchment becomes inundated with water and how the ponds, ditches and drainage channels (and roads!) can all become linked together. The first amazing occurrence after these downpours was the thousands of frogs and toads that would suddenly appear and how the air would quickly fill with a cacophony of noise, presumably as they tried to attract mates. But intriguingly for me, it was the sight of small fishes, including zebrafish, leaving the drainage ditches and ponds and spreading out across the flooded fields to take advantage of new feeding grounds and to spawn amongst the grasses before the water receded once again. No wonder every pond, ditch and stream seemed to be full of so many different fish species. This ability to move freely and colonise new areas could surely only be amplified in the monsoon season that floods much of the country every year from June to October. I remember Professor Wahab had told us that many of the un-educated people of Bangladesh had a belief that with the rains come new fishes and it was clear to see from one big rain storm we had witnessed how this belief may have originally come about. Unfortunately, this belief has lead to fish species being heavily overfished and many of the major river systems in Bangladesh are now devoid of the larger species that local people use to rely upon for food.

To get just a few more sites for our fish collections, Professor Zoarder had kindly arranged for us to visit some fairly remote rural villages several kilometres away from BAU that were only reachable by car. His



colleague/friend Monjural lived in one of these villages called Boira Salakandi and had already been checking ponds in his village for us to see if they contained zebrafish. Fortunately for us, he had found two such ponds that did. Driving away from the 'bubble' of BAU and all of its students, we entered an even more rural looking Bangladesh where life really was all about working the land - every village member seemed to be out doing something. The narrow roads/tracks that we drove down became less and less visible as they became hidden under deeper piles of freshly cut rice leaves/stems, a waste produce from the main crop that the locals dried to become fodder for their animals. These roads/tracks were one of the few clear and relatively flat areas elevated from the wet fields that made them ideal for drying their crops despite the fact that we had to drive over them, but then it was clear that not many cars came this way! The rice itself was also spread out on the floor but in huge squares next to people's homes and we watched as woman with bare feet shuffled up and down the rice to spread it thoroughly before drying. Monjural had to frequently stop and encourage cows lying in the roads to move and stop children playing on the back of our car as we trundled along at a slow speed. It was fascinating to watch and almost a shame when we came to a stop as we had reached our destination, a large perennial pond, 50 metres by 50 metres in area and deeper than most ponds we had collected from so far.

Within a very short space of time, and with tens of onlookers, we had quickly caught the most zebrafish we had from any of our sites so far (over 100), along with many other fish species that we were now getting used to seeing in our hauls. We collected the rest of the information we required to build up a habitat profile of the pond and then, although keen to get the fish back to the coolness of our aquarium at BAU, we were kindly invited into Monjural's home. We sat in the main room, which also doubled as a bedroom and storage for some of their rice which filled the space, and more, under their bed. We were immediately offered delicious looking cakes made from the rice, thirst quenching coconut milk which we drank straight from the coconut itself, and warm milk, not quite so thirst quenching in the 100 degree heat but still it was great to experience traditional Bangladesh life.

We sampled two more sites with Professor Zoarder, this time travelling to a village called Gopalpur and two separate ponds, the first a very large pond (too big to measure) which was used for growing rice and the second a smaller pond about 25 metres by 25 metres used for fish farming. We caught lots of fish in these two ponds owned by Professor Zoarder's friend Mintu and the zebrafish in the smaller pond were clearly benefitting from the food being used to feed the Indian carp that were being farmed for food as they were the largest zebrafish I have ever seen, even bigger than our laboratory ones. Again, after these collections, we were welcomed back into the homes of the local people to sample their food. What we came to realise was that, despite many of these rural people appearing to have very little in terms of worldly possessions, they were extremely warm and friendly and would always offer you something.

That brought to an end to our fish collecting adventures and all that was left to do now was to identify the various fish and plant species that we had collected along the way. Professor Karim from the Department of



**Figure 6. Bangladesh life. Top row: A typical meal just for Charlie and I served twice a day at our guest house and a view of our guest house and gardens. Second row: Fisherman up early on the old Brahmaputra at the bottom of our garden which also had the odd visitor – a monitor**

**lizard. Third row: Wild dogs off somewhere! Cycle rickshaws were the commonest mode of transport. Fourth row: Even in the dry season flooding is common but this doesn't stop the children from playing. Fifth row: Driving into the villages in search of zebrafish wasn't easy as the roads would disappear under rice fodder being dried for cattle feed. The rice itself was being walked upon by bare-footed colourfully-dressed local women to spread it out for drying.**

Agronomy had kindly offered to identify the plants for me in exchange that I would send him some of my pictures for his own references, something I still must do! We spent a few hours going through his reference books and, with his knowledge, we managed to identify well over 20 different species of aquatic, semi-aquatic and terrestrial plants that typically adorned the banks and waters of the zebrafish habitats we had sampled. We then took an example of each of the different fish species we had caught along the way to the laboratory of Professor Mostafa and Nahid and, with them, poured through identification book after identification book trying to work out what we had caught. Some species were very easy for them to identify but the differences between other species, often as subtle as a difference in the position of a spot or the number of scales on a particular part of the fish's body, even put their expertise to the test. After many hours and much discussion, we managed to identify all of our catch, from predatory snakehead species to spiny eels and see-through glassfish! All in all, Charlie and I had found an amazing 23 different fish species living alongside our beloved zebrafish and we were sure with more time and more sites to visit we could have found a lot more.

Throughout our 4 weeks, I had got on famously with Nahid, through mainly a result of our common interest in all things fishy. As a result, Nahid had regularly welcomed me into their laboratory every time they had returned from another fish collecting adventure to see what they had caught and occasionally help with their identification. Likewise, if I had returned from one of our zebrafish collections with something I thought he would be really interested in then I would immediately take it to his lab and we would share in the excitement of trying to identify it together. With such mutual enthusiasm for fish, Nahid had also invited Charlie and I to join them early one morning to catch fish from the old Brahmaputra River just near to our guesthouse. Although this wasn't home to zebrafish as it was too fast flowing compared to the still and/or slow moving and often heavily vegetated ponds and ditches we had found zebrafish in, it was still exciting to see what species we might catch. They were using this stretch of River for a fish biodiversity project, and had put in a number of reserve areas using sticks and woven baskets for the fish to use as a refuge, although it was clearly difficult to prevent people from fishing next to the reserves. In fact, they couldn't get any closer to the reserve areas if they tried! It even made me recall the day Charlie and I were working up at the field station and some local people suddenly appeared, sat on the BAU wall and cast their lines to fish into one of the experimental ponds. A funny sight at first but only if it wasn't your experiment. The sad truth was it showed the difficulties some people had in finding food for themselves and their families. On this glorious morning on the Brahmaputra we caught everything from puffer fish to beautiful banded loach, the latter of which I recall



thinking would cost you a fortune in a pet shop back home in the UK, but it was much nicer to appreciate in its natural surroundings.

During our stay, Nahid and Professor Mostafa had also kindly invited us to visit a number of fish farms (tilapia and *Pangasius*) along with their very interesting guest, Professor Terrence Tiersch who had travelled from Louisiana State University in America to give a talk about his work on sperm cryopreservation and its possible application for aquaculture and fish conservation here in Bangladesh. Professor Wahab had also taken Charlie and me on a tour of a number of fish farms. Apparently our presence at BAU had been causing such a stir with the undergraduate students that he felt we should join the third year students studying for their Bachelor of Science degrees in Fisheries on one of their day trips to meet them, chat with them and encourage them about their work. This turned out to be a brilliant and hilarious day as Charlie and I were constantly being asked by the students (of which there were at least 50) to have our photos taken with them and even to sign our autographs or write them an epilogue or two – we had finally found fame and were both loving it!

Away from work, we had also enjoyed the hospitality of the people we had met along the way. We had seen how the students lived three to a room in their university accommodation, asked question after question about our opinions on such matters as world politics, sport and inevitably women, we had been cooked for by both students and staff and taken out for numerous meals to ensure that we had sampled as much of their local cuisine as possible and that we could eat without the need for a knife and fork! What none of them realised was that we were being incredibly well fed and looked after by Mukul and Riaz back at our guest house, with three cooked meals a day. For lunch and dinner we would always be given six plates of food between the two of us and these typically consisted of a huge bowl of rice (apparently half a kilo is consumed per adult per day and you could see this from the portions we were given!), a bowl of dahl, one whole fish (usually tilapia or catfish) or prawn dish, one chicken or mutton dish, one vegetable dish and always a plate of sliced cucumber - very, very filling! People in Bangladesh had joked to us that rather than stopping work to have some food, they had to stop from eating to do a bit of work! It was so true, and in fact, and what we never expected from this trip, Charlie and I were beginning to put on weight! Being the sporty types that we are, we decided that the only answer (couldn't possibly eat less!) was to don our running shoes and go for an early morning run before breakfast at least every couple of days. This turned into a great opportunity to explore the area and see what was going on around us, albeit it was the sight of two gangly white men running in shorts that would attract much of the attention. Every patch of grass or clearing we passed was being used by groups of kids and adults for cricket and football matches and it was not long before we were spotted and asked to join in. Charlie and I didn't need a second invitation as we were both keen football and cricket players back at home and we would return on many an evening after work to play for an hour or two before retiring exhausted back at our guest house to be looked after by Mukul and Riaz, it was brilliant and did we want to come back home?

During my 4 weeks in Bangladesh, I had learnt a great deal about the richness of the wetland habitats that zebrafish occupy in the wild and that this

was a far cry from the barren and sterile tanks they find themselves being kept in back in research laboratories around the world. My knowledge gained ranged from the vast array of other fish species zebrafish shared their habitats with to the often numerous plant species and, importantly, the transitory nature of each habitat with the ability to move from ditch to pond to stream to flooded field after a heavy downpour. Even those fish that may suddenly have found themselves in an empty freshly harvested rice paddy field wouldn't have to wait long before they could move to richer waters once the rains arrived, and the rains fell often here, even in the dry season! With this new found knowledge into how zebrafish live in the wild, I had made the first and most vital step (understanding more about the biology of the species being kept) into trying to address the mission of my fellowship, that of improving the welfare of this species in the laboratory, and I returned to the UK and the University of Exeter buoyant with ideas that I couldn't wait to start on.

### **Summary of findings in Bangladesh**

- 1) Zebrafish were abundant in many shallow ponds, ditches and small streams, but not in faster flowing rivers
- 2) Zebrafish habitat is highly transitory – constantly changing and intrinsically linked to patterns of rainfall
- 3) Zebrafish can often move to colonise new habitats after heavy rainfall
- 4) Zebrafish habitat contains a rich diversity of other fish species – some predatory to zebrafish but many non-predatory that live in harmony together
- 5) Zebrafish habitat contains a rich diversity of aquatic and semi-aquatic vegetation - undisturbed ponds and ditches were full of plants including many species that grew on the water's surface, many species that grew from the bottom of the ponds/ditches and species that grew on the bank sides that tumbled in
- 6) Zebrafish were also common in disturbed habitats such as rice paddy fields and ponds for fish farming, the latter typically had less vegetation when in use but plants quickly re-colonised when left even for a short period of time
- 7) Native habitat of zebrafish is extremely rich and a far cry from the bare and sterile tanks where they are kept in scientific laboratories
- 8) Samples taken during this trip for future analyses will provide information on zebrafish population structure – sex ratio, age classes and on other habitat conditions such as more detailed water parameters

**Figure 7. Monjural, our helper, netting zebrafish for us from his home pond.**

