DOCTOR OF PHILOSOPHY

The use of biogeochemical tags to determine the origins and movement patterns of fishes

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Chapter 1

General Introduction

1.1 Migratory patterns:

Where do they originate from and where do they go too?

It is well known that some fish species undertake complicated and varied movement patterns between very distinct water bodies during their life cycles (Cadrin *et al.*, 2013), with movements confined to wholly freshwater or marine environments or transitional between the two when studying diadromous species. For fisheries management, conservation and exploitation purposes it is fundamental that we understand their ecology and movement patterns during their life time if we are to utilize them sustainably. Freshwater species such as resident brown trout *Salmo trutta* can undertake short migrations of a few kilometers within streams for feeding or to find areas suitable for spawning (*e.g.* Vøllestad *et al*., 2012). In contrast species such as the paddlefish *Polyodon spathula* are known to be highly mobile making extensive movements (*ca.* 2,000 km) within river systems and are frequently capable of covering large distances (*ca.* 40 km) within a 24 hour period (Jennings and Zigler, 2000). One of the longest freshwater migrations observed is the Amazonian catfish *Brachyplatystoma rousseauxii*. Migrations of these species regularly exceed 10,000 km between juvenile nursery areas in the estuary of the Amazon to breeding zones in the head waters of the western Amazon basin close to the Andes (Garcia Vasquez *et al.*, 2009).

Marine species too have variable migratory patterns, with large-scale movements in the order of 100s of km between nursery, feeding and spawning grounds not uncommon. North East Atlantic examples of these include Atlantic herring *Clupea harengus* (Ruzzante *et al.*, 2006), European plaice *Pleuronectes platessa* (Dunn and Pawson, 2002) and European sea bass *Dicentrarchus labrax* (Pawson *et al.*, 2007). Some fishes however, undertake transoceanic migrations covering several 1000 km's, such as the feeding migrations of salmonids while at sea (Groot and Margolis, 1991; Hansen and Quinn, 1998). Greater migrations can be observed for the European eel *Anguilla anguilla* which undertakes migrations of *ca.* 5,000 km from its feeding grounds in European freshwaters to spawning grounds in the Sargasso Sea (Aarestrup *et al*., 2009). The marine species which undertakes the longest transoceanic migration is probably the white shark *Carcharodon carcharias*, with one individual recorded as undertaking a migration from South Africa to Australia and back, a journey in excess of 20,000 km (Bonfil *et al.*, 2005). Evidently, both marine and freshwater fishes undertake movements of

considerable magnitude during differing stages of their life cycles. The main questions facing both managers and scientists alike are where have those fish come from? (*i.e.* natal origin) and more importantly where and why do they go? (*i.e.* movement patterns).

1.2 Tracking migratory patterns:

Can we determine these movement patterns?

The terrestrial movement patterns of animals have been easier to understand and track due to their coexistence within the same environment as man. However, this is not as easily undertaken when trying to observe the movement patterns of aquatic animals. By the nature of the medium in which they live – water, compounded by the vast three dimensional environments of the oceans, we as humans cannot easily observe where they go to and where they come from. Despite these difficulties, attempts have been made to track movement patterns and identify origins of fish as early as the 1880s using external tags attached to the fish (Loerke & Cadrin, 2007). There are two methods readily used in trying to track movement patterns of fish, the use of "applied" markers where artificial tags are introduced to the fish by researchers and "natural" markers where unique natural characteristics are used in identifying origins of the individual fish and used to observe movement patterns (see Cadrin *et al.*, 2013 for a detailed review of the subject area).

Applied tags can be either placed on the external surface of the aquatic animal allowing the tag to be visually identified if recaptured or implanted within the body (*i.e.* PIT tags in fish) and vary dependent upon species, time and more importantly funding, with some types of tags costing nothing *e.g.* notching / fin clipping, up to a few thousand pounds each *e.g.* satellite tags. For aquatic animals tags include: V-notching (in crustaceans) or fin clipping (in fishes), polyethylene or rubber ribbons / discs (in shellfish), visible implant elastomer (VIE, in fishes), anchor tags (in fishes), laminated disc tags (in flatfishes), passive integrated transponders (PIT tags, in fishes), acoustic tags (in fishes; used in radio-tracking), archival data storage tags (DSTs, in fishes) and "pop up" satellite tags (in fishes) (see Cadrin *et al.*, 2013 for a detailed review of the range of applied and natural markers used in fish biology).

The use of conventional tag-recapture and radio-tagging of individual fish has assisted in reconstructing the movement patterns of various species (see reviews by

Cooke *et al*., 2011 and Cadrin *et al.*, 2013). These methods are conducted frequently by Government fisheries agencies monitoring fish stocks and management programmes *e.g.* large-scale tagging programmes in the UK on European plaice (Dunn and Pawson, 2002) and European sea bass (Pawson *et al.*, 2007) by Cefas to inform ICES on management policies. However, these tagging programmes are extremely labour intensive and logistically difficult to implement (tag and recapture rates can be very low see King, 2007; *e.g.* see Herzka *et al.*, 2009). Furthermore, cost can be high depending upon the number of samples to be targeted and the equipment used *(i.e.* radio tags, monitoring equipment), with financial constraints reducing the number of fish to be monitored and other costs incurred due to lost or damaged equipment (*e.g.* loss of expensive radio satellite tags), combined with poor returns from recaptured or successfully tracked fish (Cadrin *et al.*, 2013). More so, we must consider the gains in our knowledge from using successful tagging programmes when comparing costs incurred for time and equipment (*e.g.* Block *et al.*, 2001; Bonfil *et al.*, 2005; Galuardi and Lutcavage, 2012). Methods using tags such as these have increased our understanding of homing traits observed for anadromous adults to their natal rivers which have proved difficult in the past using many conventional tagging methods.

Some success can be attributable to more advanced technology using archival and satellite radio tags (Dingle, 1996; Elsdon *et al*., 2008). Miniaturized artificial radio tags combined with more sophisticated radio telemetry have enabled "tiny" PIT tags to be developed that have been used to document behaviour in ants (Moreau *et al*., 2011) and bees (Decourtye *et al.*, 2011), with tags of *ca.* 6mm in length used on studies of small zebra fish (size range 16-42 mm; Cousin *et al.*, 2012). However, cost and the use of many conventional external tags have hindered our understanding of natal origins and early movement patterns of juvenile fishes due to size limitation *i.e.* high mortality observed for small fish during the tagging process (see review in Cadrin *et al.*, 2013). This problematic subject is therefore only feasible when the fish have attained a size at which the impact of attaching / implanting the tag will no longer affect their survival or growth (*e.g.* Ombredane *et al*., 1998; Richard *et al*., 2013). However, such an understanding of the early life history patterns is fundamental if we are to further understand their population structure and movement dynamics (Kennedy *et al*., 2002; Metcalfe *et al*., 2002).

The understanding of movement patterns and life history strategies is not only confined to juvenile fish. Tracking and monitoring of adults within the marine

environment too can have its problems using traditional tagging techniques. The number of fish required to be tagged and the time required to get meaningful results to elucidate stock structure and movement patterns can be immense (*e.g.* 30+ years monitoring programme on the European sea bass in UK waters. Holden and Williams, 1974; Kelley, 1979; Pawson *et al*., 1987; Pawson *et al.*, 2007; Pawson *et al.*, 2008; Quayle *et al*., 2009).

1.3. Natural markers

An alternative approach to the sometimes costly and time consuming use of artificial tagging in both adult and juvenile fish in helping to understand geographical distributions, dispersal and movement patterns has been the use of natural markers (Walther and Thorrold, 2009; ICES, 2012). Natural markers come from a variety of applications, and can be used to look at movement patterns and help in identifying origins of an individual animal using one or more of their unique natural characteristics. These natural markers have included the use of parasites, bacterial communities, distinctive body markings, meristics / morphometrics, genetics and the use of chemical tags such as stable isotopes and trace element microchemistry.

Parasites have been used as a tool for stock discrimination of both demersal and pelagic marine fish species as well as anadromous salmonids, cetaceans and invertebrate species (see Mackenzie and Abaunza, 1998; Mackenzie, 2002). Their use as biological markers in identifying origin or movement has also been studied. Mackenzie and Abaunza (1998) and Mackenzie (2002) showed where parasitic infection had been found to be endemic to a specific geographical region; animals which were subsequently caught outside that region but were infested with these site-specific parasites had at some point visited that area during part of their life history. Similar studies using bacterial populations which are associated with the mucus layer of fish and surrounding seawater have been used to infer origins in gadids (Wilson *et al.*, 2008; Smith *et al.*, 2009) and on a more global scale in tracing origins of marine ornamental fishes (Cohen *et al.*, 2013).

Another method used in the identification of individuals in some aquatic species and to track their movement is the use of natural body markings such as tears, marks, notches and scars in fins and tail flukes, and spot patterns. Mainly adopted for the use with marine megafauna *e.g.* large elasmobranchs (Castro and Rosa, 2005; Van Tienhoven *et al*., 2007) and cetaceans (Dufault and Whitehead, 1995), this approach has also been

used in identifying small juveniles in some species like salmonids (Leaniz *et al.*, 1994; Donaghy *et al.*, 2005; Merz *et al.*, 2012).

One extensive method used in understanding population structure is the use of genetic markers, with these markers used for population differentiation (Hamilton, 2009; Nielsen and Slatkin, 2013), stock discrimination for fisheries management purposes (Carvalho and Hauser, 1994; Shaklee *et al*., 1999) and to identify and conserve species of high genetic pedigrees (Avise, 1989; Hedrick, 2001). Changes in how genetic studies were conducted have enabled a more precise understanding of stock structures, with studies using protein polymorphism giving way to the use of molecular DNA polymorphisms using microsatellites and mitochondrial DNA (Begg and Waldman, 1999; Okumuş and Ciftci, 2003). However, the standard tool for the use in assessing population variation in fishes for the use of exploitation and conservation is the use of nuclear DNA (*e.g.* Sato *et al*., 2004). One group of fish whose population structure and genetic diversity has been extensively studied are the salmonids (*e.g.* Sato *et al*., 2004; Verspoor *et al*., 2007). Studies using genetics have looked at both large geographical and spatial scales (*e.g.* Utter *et al.*, 1989; Bernatchez *et al.*, 1992; King *et al*., 2001; Sato *et al*., 2004) to the smaller fine-scale populations observed within a single river catchment (*e.g.* Carlsson and Nilsson, 2000; Kitanishi *et al*., 2009; Stelkens *et al*., 2012), with very good results.

1.4. Biogeochemical markers

The use of natural biogeochemical markers in animal tissues and hard parts (*e.g*. scales, fin rays and otoliths) have provided research scientists with alternative marking methods for species which have been previously difficult to tag using conventional techniques (Walther *et al*., 2008). One method growing in interest to study the origins and movement patterns of animals is the use of stable isotopes (Rubenstein and Hobson, 2004: Graham *et al.*, 2010; Hobson *et al.*, 2010; Trueman *et al.*, 2012; McMahon *et al.*, 2013). Using differences observed from the isotopic composition of water (West *et al.*, 2010) isotopes such as nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$), oxygen ($\delta^{18}O$), sulphur ($\delta^{34}S$) and strontium ($\delta^{87}Sr$) have been used to infer origins and movement patterns of fish within freshwater, marine and estuarine environments (see Kennedy *et al.*, 2005; Barnett-Johnson *et al*., 2008; Zeigler and Whitledge, 2010; Trueman *et al.*, 2012; Martin *et al.*, 2013; McMahon

et al., 2013). Most studies of this nature have tended to examine large scale movement patterns within the marine environment (*e.g*. bluefin tuna *Thunnus thynnus*, yellowfin tuna *Thunnus albacares* and swordfish *Xiphias gladius*; Ménard *et al*., 2007; Rooker *et al*., 2008; Graham *et al.*, 2010). However, interest in studying the movement patterns of fish within the freshwater environment through the application of these isotope markers is growing (*e.g.* Kennedy *et al.*, 2005; Barnett-Johnson *et al.*, 2008; Zeigler and Whitledge, 2011; Martin *et al.*, 2013). One such application using brown trout *S. trutta* scales and the isotopes $\delta^{13}C$ and $\delta^{15}N$ enabled brown trout to be classified back to their site of origin within the River Dee catchment in North Wales with a high degree of accuracy (Ramsay *et al*., 2012).

Since the 1970's interest in the use of calcified structures and their chemical composition to address wide ranging questions in fisheries science has grown. This concept assumes the chemical composition of these structures reflects broadly the physio-chemical characteristics of the environment to which those fish were exposed (de Pontual and Geffen, 2002). One such application proving to be an extremely valuable tool for studying movement and life history characteristics of teleost fishes is the use of microchemistry, more specifically the use of calcareous structures such as fin rays, vertebrae, fin scales and otoliths (*e.g.* Wells *et al.*, 2003*b* Clarke *et al.*, 2007; Elsdon *et al.*, 2008; Ramsay *et al.*, 2011; Tillett *et al.*, 2012). One of the more commonly used calcified structures in microchemistry studies, fish otoliths, have been often referred to as a continuous recorder of environmental exposure (Campana *et al*., 1997), as their composition has one of the highest potentials to convey environmental information compared to other calcified structures (de Pontual and Geffen, 2002; Elsdon *et al.*, 2008).

Otoliths (or ear stones) are paired calcareous structures within the inner ear of fish that have been routinely used as biochronological markers for aging of fish due to the daily cyclic deposition of crystalline (*i.e.* calcium carbonate) and organic material (*e.g.* proteins: Campana and Neilson, 1985; Elsdon *et al*,. 2008) forming concentric rings over the life time of the fish (Beamish and McFarlane, 1987). However, due to the metabolically inert nature of these calcium carbonate structures (more specifically in its aragonite morph: see Degens *et al*., 1969; Mugiya, 1972; Radtke, 1978), the mineralogy of the otoliths is thought to remain unaltered after the sequential deposition of the calcium carbonate. Composed of 99% $CaCO_3$ the metabolically inert nature of otoliths, combined with trace and ultra-trace elements which make up the remaining 1% (see Campana and Neilson, 1985; Payan *et al*., 2004) are derived in some degree from the

elemental chemistry of the surrounding ambient water (Farrell and Campana, 1996; Bath *et al*., 2000; Walther and Thorrold, 2006; Jolivet *et al*., 2013). Generally considered as biological archives (Campana, 1999; Thresher, 1999; Payan *et al*., 2004; Jolivet *et al*., 2008), these unique chemical properties allow otoliths to be used as natural biogeochemical markers (Campana, 1999).

1.5. Otolith microchemistry

To understand the incorporation of trace elements into the otolith, the original chemical composition of the structure and its ability to act as a natural elemental tag in fish population studies should be described. Fish otoliths are paired structures located within the inner ear of all bony fish and used for maintaining equilibrium, processing directional cues and sound reception (Wright *et al*., 2002; Popper and Fay, 2011; Sturrock *et al*., 2012). Comprised mainly of calcium carbonate ($CaCO_3$) aragonite crystals crystallized onto a fibroprotein organic matrix (Dannevig, 1956; Degens *et al*., 1969; Campana & Neilson, 1985; Wright *et al*., 2002; Tohse & Mugiya, 2002; Payan *et al*., 2004), otoliths grow continuously throughout the life history of the fish from the early embryonic stages to the time of its death (Campana & Neilson, 1985; de Pontual and Geffen, 2002; Elsdon *et al.*, 2008). Studies conducted on a number of broad elemental assays have shown that otoliths are relatively pure, with total inorganic impurities comprising less than 1% of the total otolith weight (Edmonds *et al*., 1992; Thresher *et al*., 1994; Proctor *et al*., 1995; Campana *et al*., 1997). A comprehensive review by Campana (1999) indicated a total of 31 major (Ca, C, O and N), minor (> 100 mg kg^{-1}) and trace (< 100 mg kg^{-1}) elements have been detected in otoliths, with otolith elemental composition mainly dominated by calcium (Ca), oxygen (O) and carbon (C), with other elements such as Na, Sr, K, S, N, Cl and P present at concentrations > 100 ppm, and the so-called "trace" elements at concentrations < 100 ppm. However, with the improvement in analytical detection limits during the last decade the number of detectable elements in larval and adult fish otoliths has increased to 50 (Chen and Jones, 2006; Sturrock *et al*., 2012).

Analytical techniques used to assess the elemental composition of fish otoliths require the $CaCO_3$ polymorph to be in its normal crystal morph which for the sagittae and lapilli otoliths is in the form of aragonite. However in some circumstances this morph can be replaced (often only partially) by vaterite (or more rarely calcite) to produce what are

known as “crystalline” otoliths (Gauldie *et al*., 1993; Wright *et al*., 2002) which differ in their chemical uptake of the minor and trace elements. de Pontual (unpublished data, cited in Wright *et al*., 2002) showed decreases in concentrations of Sr, Na and K in the vaterite portion of the otolith, with Mg and Ca showing higher concentrations than observed within the normal aragonite otolith. Brown and Severin (1999) also reported a decrease in concentrations of Sr, Na and K within vaterite otoliths, but did not report any findings on Mg concentrations. Studies have shown that the otolith is impregnated with the endolymph (a liquid with special viscous properties; Wright *et al*., 2002) in which it bathes (Gauldie and Coote, 1997; Gauldie and Cremer, 1998; Milton and Chenery, 1998; Proctor and Thresher, 1998). Furthermore, Thresher (1999) has commented that the fluid component of the endolymph is a major influence in determining the ultimate composition of the otolith as the calcium carbonate precipitates out from the endolymphatic fluid (Miller *et al*., 2006). Wright *et al*., (2002) found that the principal components of the endolymph display decreasing or increasing proximodistal concentration gradients that may act as a driving force in the biomineralization processes, enabling heterogeneous distribution of some elements on the otolith surface.

The endolymph proteins have also been recognised as playing a pivotal role in the uptake and inhibition of calcium carbonate as well as other processes of biomineralization and organomineralization (Trichet and Defarge, 1995). Campana (1999) has described how the calcification process of the otolith is heavily dependent upon the composition of the endolymphatic fluid surrounding it and concludes that one of the key physical regulating factors of the endolymph is pH, which is determined by the concentration of bicarbonate ions within the endolymph (bicarbonate is one of the ion products of carbon dioxide in solution; Romanek and Gauldie, 1996; Payan *et al*., 1997). Alkalinity within the endolymph is regulated by proton secretion through the saccular epithelium, which reduces the rate of calcification (Payan *et al*., 1997).

However, few studies have been conducted on the endolymph fluid and the organic components of the otolith, and additional studies are therefore critical if we are to fully understand the processes of otolith formation and composition (Wright *et al*., 2002). However, the presence of a pH gradient within the inner ear of teleost fish and its relationship to biocalcification rates (see Gauldie and Nelson, 1990; Gauldie *et al*., 1995; Payan *et al*., 1997) through a pH-dependent proton secretory mechanism, may adversely affect the patterns of elemental deposition through changes in endolymph pH during periods of trauma (*e.g*. stress: affecting blood pH and in turn affecting endolymph pH).

1.5.1 Otolith elemental assimilation

The processes involved in otolith formation are unlike those of biomineralization of molluscan shells and coral skeletons in that the epithelium of the otolith is not in direct contact with the region of calcification or with the surrounding water. Indeed, the process of calcification can be described to a certain extent as being based on purely physical principles being acellular and heavily reliant upon the composition of the endolymphatic fluid that surrounds and bathes the otolith (Campana, 1999; Wright *et al*., 2002; Payan *et al*., 2004). The incorporation of environmental factors into the composition of the otolith is a complex process which according to Wright *et al*., (2002) involves four nested compartments: (1) the external medium, where variations in abiotic factors occur; (2) the blood plasma, which is respondent to the external medium and also exhibits endogenous variation; (3) the endolymph, which modulates the various signals and regulates the formation of the otolith; and (4) the otolith, which integrates and records responses to all these signals.

The basic pathway for the uptake of inorganic elements into the otolith is from the surrounding medium (water) into the blood plasma via the gills or intestine. These are then transported into the endolymph and finally precipitated into the crystallizing otolith (Campana, 1999; Wright *et al*., 2002, Miller *et al*., 2006). In freshwater fish, branchial uptake (via the passing of water over the gills) is the primary source of elements, while the main source of water-borne elements for marine fish is through the continual action of drinking water, which is then absorbed through the intestinal epithelium as part of their osmoregulatory process (Simkiss, 1974; Olsson *et al*., 1998; Wright *et al*., 2002). There is, however, a small, unknown fraction of elements that can be incorporated into the blood plasma via the fish's diet through the digestion of food (*e.g.* Sr), (Farrell and Campana, 1996; Gallahar and Kingsford, 1996). However, the rate of assimilation of minor elements into the otolith in this manner is considered minimal according to a study by Hoff and Fuiman, (1995).

1.6. Otolith microchemistry: Metabolically inert

It has been assumed that the otolith is metabolically inert and acellular in nature and that its overall composition is not subject to dissolution, reabsorption or erosion, however, this

assumption has been questioned (Campana and Nielson, 1985; Gunn *et al*., 1992; Campana, 1999; Hedges *et al*., 2004; Arslan, 2005; Miller *et al*., 2006). Indeed, the assumption that the composition of the otolith cannot change significantly when it is *in situ* in the fish just prior to removal or after extraction when it is subject to analysis may be incorrect (Proctor and Thresher, 1998). The possibility of micro-chemical alterations such as leaching or contamination of otoliths during the extraction process has been highlighted as a point of concern (*e.g*. Proctor and Thresher, 1998; Hedges *et al*., 2004).

The possible effects of trace elements leaching from these calcium carbonate aragonite structures prior to extraction and the possible labile nature of some Group II alkaline-earth metals (*e.g.* Mg) combined with the effects of *post mortem* handling have been little studied. The effect on otolith microchemistry of how the fish are captured, the problems associated between the time of capture and when they are dispatched (as reported from a study on deep sea fish species; Longmore *et al*., 2011) and the time which they are stored frozen (*e.g.* days – months – years) prior to otolith extraction have also been little studied. Some authors indicate the importance of otolith removal directly over storage of the whole fish carcasses (prior to otolith removal) or the storage of the otoliths themselves (see Milton and Chenery, 1998; Rooker *et al*., 2001; Brophy *et al*., 2003; Swan *et al*., 2006) due to the possible chemical composition being compromised after the capture of the fish (Proctor and Thresher, 1998; Hedges *et al*., 2004).

1.7. Otolith microchemistry: Temporal stability

One of the main applications of otolith microchemistry has been to distinguish between water masses (both between and within freshwater, estuarine and marine water masses) that differ in their chemistry over spatial scales (Gillanders and Kingsford, 1996; Thorrold *et al*., 1998*b*; Gillanders and Kingsford, 2000). These studies have focused on the movement patterns of fish between estuarine and marine waters (Gillanders, 2005) and in observing anadromy movements of fish from freshwater environments to marine (Kalish, 1990). However, interest in the movement of fish in freshwater and their migration to estuarine / marine environments is growing (*e.g.* Kennedy *et al*., 1997; Walther and Thorrold, 2009; Ramsay *et al*., 2011; Veinott *et al*., 2012). Fish visiting or inhabiting water bodies that differ in their water chemistry will incorporate trace and ultra-trace elements from the surrounding water into their otoliths, picking up what can

be described as a unique chemical fingerprint for that moment in time. If we are to use spatial differences in water chemistry as a tool to look at origins or movement patterns of fishes then we need to have confidence that observed differences are maintained over time, *i.e*. exhibit temporal stability. However, water bodies are by their very nature highly dynamic environments with elemental concentrations differing considerably over small temporal scales *e.g.* as seen in estuarine environments (Elsdon and Gillanders (2006*b*). A number of studies have attempted to address the issue of temporal stability of otolith microchemistry but with limited success (see Elsdon *et al*., 2008).

Most of these studies have tended to conduct their work over short term periods of ≤ 2 years (Edmonds *et al*., 1992; Patterson *et al*., 1999; Campana *et al*., 2000). The exception is the study by Campana *et al*. (2000) whose research examined temporal stability over a longer time frame, 4 – 13 year intervals, but their time series analysis did not use a continuous data set with 4 locations resampled 5 times over a 13 year period. Milton *et al*., 1997 found that temporal stability may have been a confounding issue when undertaking a study to identify the spawning estuaries of the tropical shad (*Tenualosa toli*) around the coastal seas off Sarawak and that further studies conducted over a greater time frame were required. Similarly, Walther and Thorrold (2008) using geochemical signatures contained within otoliths of an anadromous fish (American shad, *Alosa sapidissima*) found significant inter-annual variability between several rivers when looking at otolith $\delta^{18}O$ values. Few studies have attempted to look at the effects of inter-annual and intra-annual variability using biogeochemical analyses (Mercier *et al*., 2011; Sturrock *et al*., 2012). Clearly given the importance of understanding possible temporal effects and the stability of those chemical tags used in otolith microchemistry as a tool to understand movement patterns and origins of fish, more work is required to clarify and validate the use of this technique.

1.8. Otolith microchemistry: A tool to delineate between nurseries

For many marine fish species, the juvenile and adult phases of the life cycle can occupy very different environments that are geographically separate from each other (Brown, 2006*b*). Previous researchers have examined whether this has resulted in distinctive otolith elemental signatures for these different geographical areas that can be used to identify the origins of fish and thereby study their movement patterns and population

connectivity (Gillanders and Kingsford, 1996; Campana *et al*., 1994; Gillanders and Kingsford, 2000; Geffen *et al*., 2003; Gillanders, 2005; Brown, 2006*b*; Vasconcelos *et al*., 2007; Elsdon *et al*., 2008).

The changes in concentrations of various trace elements within the environment (*e.g*. marine / estuarine and freshwater or from food such as prey items) can influence the elemental concentration observed not only in the body tissues of the fish being studied, but also the concentrations that are incorporated into the calcium carbonate aragonite matrix of their otoliths (Campana, 1999; Geffen *et al*., 2003; Gillanders and Kingsford, 2003). During the growth of the fish, elements contained within the water mass that surrounds the fish during its life are deposited within the structural layers of the otoliths. The uptake of the inorganic elements into the otolith stems from the surrounding medium (water), passes into the blood plasma via the gills and / or intestine and are then finally transported to the endolymph fluid which surrounds the crystallizing otolith (Campana, 1999; Wright *et al*., 2002; Brown, 2006*b*). The incorporation of the various elements into the otoliths that occurs via the binding process of ions to proteins or assimilation between the spaces of the calcium carbonate and protein matrices (Gillanders and Kingsford, 2000) allows these structures to act as a long term data record of the environment(s) experienced by the fish. This has given rise to the use of microchemistry as a tool to try to identify the different geographical areas visited by the fish during its life, or to distinguish between fish reared on different nursery grounds and to identify the natal origin of fishes: all of these approaches having clear conservation and management implications (Geffen *et al*., 2003). Furthermore, the use of otolith biogeochemistry has enabled scientists to differentiate between fish populations residing in adjacent freshwater catchments and fish which reside in different tributaries within a catchment during some part of their life history (*e.g*. Kennedy *et al*., 1997; Walther and Thorrold, 2009; Ramsay *et al*., 2011, 2012).

The use of otolith microchemistry has enabled some fish to be to be identified to their habitat of origin with a very high classification rate using statistics such as discriminant function analysis. For example, Vasconcelos *et al*. (2007) used the otolith chemical signatures to allocate five species of fish to their estuarine nurseries of origin along the Portuguese coastline with an accurate group classification rate of 77.1%, indicating the possible applications for this type on analysis in assigning adult fish to their estuarine nursery and in understanding connectivity between nursery grounds and coastal areas. A recent study by Fodrie and Herzka (2008) used otolith microchemistry to

identify movement patterns of juvenile flatfish and the contribution of nursery grounds within coastal embayments of southern California and Baja California, Mexico, with an accurate group classification rate between inner and outer zones of 61%, indicating the coastal embayments possessed distinct chemical fingerprints. Leakey *et al*. (2009) looked at the multi-elemental chemistry of juvenile sole (*Solea solea*), whiting (*Merlangius merlangus*) and sea bass in the Thames Estuary and identified differences in otolith chemistry between estuarine and coastal-captured fish. The differences between estuarine and coastally-caught fish were more evident in the less mobile sole compared to the more active whiting and bass. However, due to the physiological ability of the bass to migrate further up the estuarine environment, chemical signatures could be distinguished between the estuary-caught fish when looking at the salinity gradient change using Ba: Ca data.

Not only can the concentrations of naturally occurring elements within water masses (marine, estuarine and freshwater) be used to identify the origins of fishes, elements derived from anthropogenic activities can also be used to help distinguish between fish from different areas. For example, Geffen *et al*. (2003) studied the spatial differences observed in the otolith trace element concentrations of whiting (*Merlangius merlangus*) and plaice (*Pleuronectes platessa*) from five sites in the eastern Irish Sea (plaice) and from the eastern and western Irish Sea (whiting). Their results indicated that the observed spatial variation in otolith elemental concentrations were consistent with the distribution of contaminants observed around the Irish Sea by an earlier study by Leah *et al*. (1991, 1992*a*, *b*).

Further studies have shown that not only can the elemental composition of fish otoliths indicate regional signals but also the stable isotopic composition of the otoliths and scales can also indicate spatial variability and can be used to identify the origins of fishes. For example, Whitledge (2009) looked at the potential of using otolith chemistry to identify source environment for fishes in the upper Illinois River and Lake Michigan and to identify individuals that may have breached or circumnavigated electrical barriers in the Chicago Sanitary and the Ship Canal or had been transferred (via bait buckets) between those formally isolated drainages. Using both elemental and stable isotope otolith chemistry he found Lake Michigan fish were distinct from individuals sampled within the Illinois River and tributaries (using otolith $\delta^{13}C$) and fish collected from both the Fox and the Des Plaines Rivers could be distinguished not only from one another but from fish taken in the Illinois and Dupage Rivers (using Sr: Ca and Sr: Ba ratios). A more recent study has shown the use of $\delta^{13}C$ and $\delta^{15}N$ isotopes measured from scales of *S.*

trutta were marginally superior in their classification accuracy in identifying trout to their natal site on a small spatial scale compared to using multi-elemental signals obtained from scale hydroxyapatite and otolith aragonite within the River Dee catchment in North Wales (Ramsay *et al*., 2012). The natural occurring variability observed in elemental concentrations recorded in otoliths combined with the use of microchemistry and isotopic analysis has shown increasing promise in helping researchers in identifying movement patterns over small spatial and larger geographical scales for fish within discreet water bodies. The continued use of these methods and the advances in technology can only strengthen our knowledge and understanding of fisheries movement and assist in future management of both stock and population structures.

1.9. Otolith microchemistry: Analytical techniques

The analysis of biogeochemical structures such as fish otoliths as tools to infer distinct connectivity patterns, stock structure and individual migrations for various fish species in both marine and freshwater environments has increased during the last decade (see Sturrock *et al*., 2012). In order to quantify trace elemental concentrations a number of techniques have been used with some analytical methods being preferred more over others. Techniques have included electron microprobe energy-dispersive and wavelength-dispersive analysis, proton-induced X-ray emission, graphite furnace atomic absorption and micro-PIXE analysis (Sie and Thresher, 1992; Campana *et al*., 1997; Proctor & Thresher, 1998; Campana 1999; Thresher 1999). However, the most commonly-used technique within this field of research is inductively-coupled plasma mass spectrometry or ICP-MS. This technique has grown in use partly due to its ability to detect elements at extremely low concentrations (parts per quadrillion – parts per trillion) allowing for a greater range and suite of elements to be precisely and accurately quantified (see Ludsin *et al*., 2006 and references therein).

1.10. Otolith microchemistry: ICP-MS

The use of ICP-MS offers many benefits to researchers wishing to analyse trace and ultra-trace metals in either solutions (*i.e.* water) or solids (*e.g.* soils, rocks biological material), with most of the elements contained within the periodic table measurable (ICP-MS Technical Note, 2001). The use of ICP-MS achieves higher productivity and lower detection limits when compared to other systems and allows the multiple detection of numerous elements simultaneously, allowing rapid sample processing. To help understand the concept of ICP-MS and understanding its use in fisheries research a brief overview on how samples are introduced and how the ICP-MS works is presented.

Most samples which are analysed using ICP-MS are in solution form (*e.g.* digested material or liquids) which are then introduced into the plasma. However, solid samples can be analysed using ICP-MS by using heated cells or in most cases lasers which vaporize the sample material (similar to digesting a solid sample in solution-based analysis) which is then introduced into the plasma. Samples are first introduced to the plasma via a cross flow nebulizer (*i.e.* gas flow is at right angles to the flow of the sample) using a peristaltic pump system (see Figure 1.1). The nebulizer shears the liquid into vapor droplets forming an aerosol, with the now aerosolized sample droplets (at the correct size and velocity) pass through the spray chamber and on into the plasma.

The inductively-coupled plasma is generated by passing argon gas through a series of concentric quartz tubes and is energized by inductively heating the gas with an electrical radio frequency coil, making the gas electrically charged (containing both electrons and ion concentrations) at a temperature around 6000^{o}C. The plasma gas is essentially neutral with positive ions almost all singly charged with equal amounts of both electrons and ions in the volume of the plasma. The liquid droplets containing the sample matrix and the elements we wish to assay are dried to form a solid then heated to form a gas.

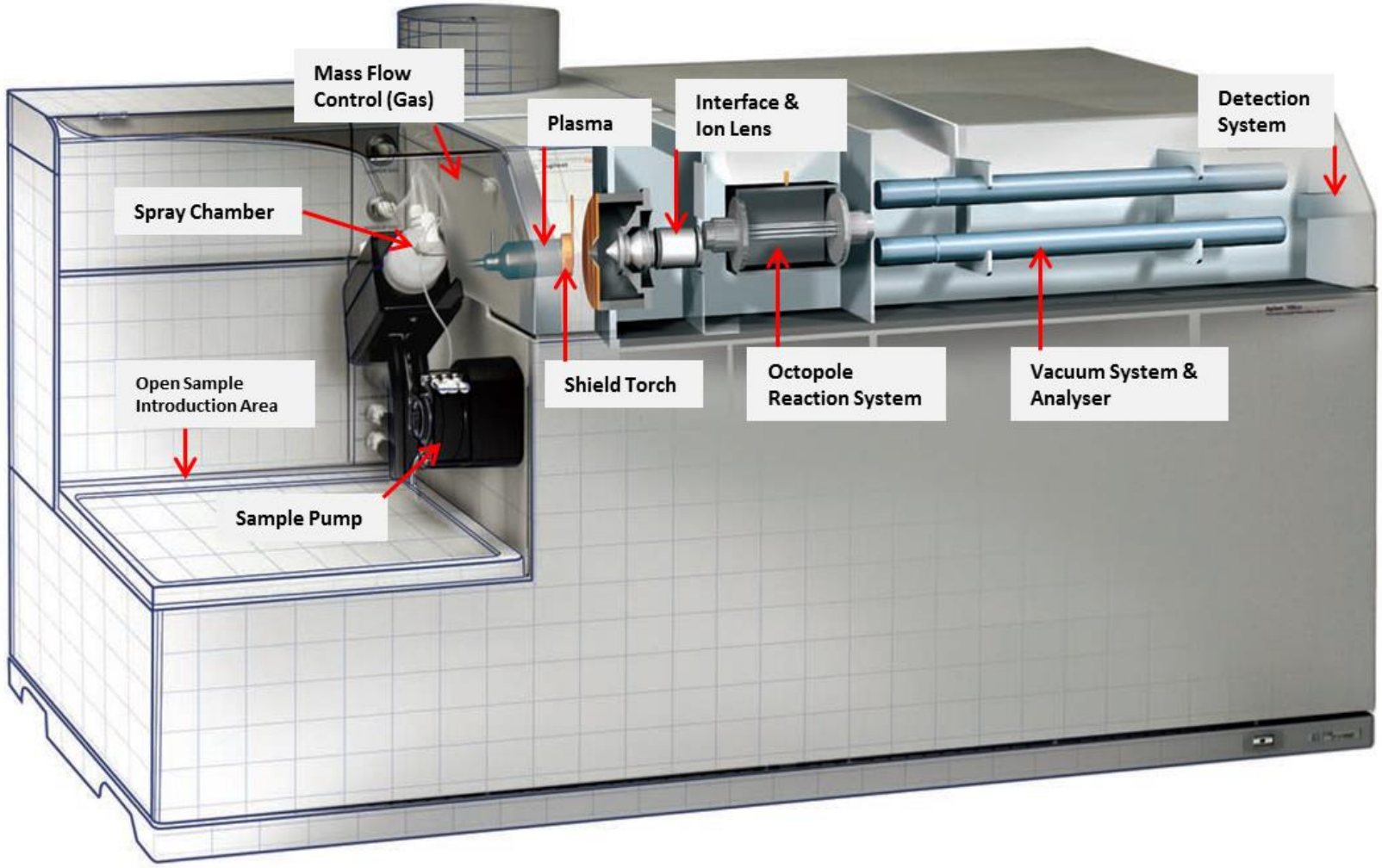

Figure 1.1. Layout of an inductively-coupled plasma mass spectrometer (ICP-MS), indicating sample introduction area and the identification of the components used in the analysis of both solution based and laser ablated material (Adapted from ICP-MS Technical Note, 2001).

The atoms travelling through the plasma absorb energy directly from the plasma and in doing so release an electron to become singly charged ions. At this point the singly charged ions enter the inter face region (Figure 1.1) where two cones (sampler and skimmer) each having an opening of 1 mm in diameter at their apex, sit a few millimetres apart and channel the sample towards the detector. To prevent any ions colliding with any gas molecules a vacuum is set up between the interface (cones) and the detector using a combination of turbomolecular pumps and roughing pumps creating a pressure or around 1×10^{-5} Torr.

Ion lenses positioned directly behind the interface focus the ions from the sample matrix towards the second generation octopole (Figure 1.1). Sample ions are nearly all positively charged and as such will repel other ions. A focusing lens created by passing the ions through a charged metal cylinder allows these positively charged ions to be focused into an ion beam which then passes into the octopole (the octopole consists of 4 rods approximately 20 cm in length and 1 cm in diameter). The ions are then separated in the octopole by their relative mass (*i.e.* mass filter) using the correct combination of radio frequencies and voltages between the four rods. Ions are then sorted out using their mass-to-charge ratio (m/z), with the selected m/z allowing only one mass measurement to pass through the detector (Figure 1.1) at any given moment. Ions exiting the octopole strike the surface of the detector (a series of dynodes) generating an electronic signal which is measured using the release of an electron each time an ion strikes, starting the amplification process and creating a cascade of electrons which in turn generates a measurable pulse. This measurable pulse allows the number of ions striking the first dynode to be counted and allows the identification of those elemental concentrations found within the sample to be identified. The ion counts measured by the detector are then transferred (as counts per second, cps) to the Agilent Proprietary ICP-MS software (Agilent Technologies, U.S.A.), allowing the ICP-MS software to determine accurately how much of a specific element has been analysed in the material. This information is then extracted and subsequently transferred onto excel data spread sheets for the user to read and interpret the results.

1.11. Conclusion

The use of natural tags / markers (such as trace elements and stable isotopes) demonstrate great promise in determining movement patterns of fish populations and the possible identification of source environment for juveniles and adults that migrate or are transferred between areas. This has been facilitated by increased technological developments in stable isotopic research being more accessible (economically) to scientific researchers. The use of these chemical signatures may enable information to be gathered on the movement from specific habitats of juvenile fish and allow specific protocols for management and conservation efforts to focus on the protection of these habitats. Furthermore, studies must be focused on movement patterns to supplement data already gathered on density, growth and survival of juvenile fish in putative nursery areas enabling a greater rate of recruitment back to an adult population.

1.12. Aims of research for the thesis

The aims for the research of the current thesis are outlined as follows:

1. To determine whether pre-processing treatment of fish samples can influence the otolith microchemistry of fishes (Chapter 2). This aim will be achieved by examining how four common methods of dispatching fish (*i.e*. direct cull or using three types of anaesthetic: 2-phenoxyethanol, clove oil or MS-222), three of the most commonly used methods in transporting those fish samples (*i.e*. left fresh in air, on ice or in ice) and the period of storage duration (1, 5, 25 days and 6 and 12 months respectively) may affect the trace elemental concentrations of sea bass (*Dicentrarchus labrax*) otoliths.

2. To determine whether otolith elemental chemistry exhibits long-term temporal stability (Chapter 3). This aim will be achieved by examining intra-annual (*i.e.* within year) and inter-annual (*i.e*. between years) stability in the trace element chemistry in otoliths of juvenile plaice (*Pleuronectes platessa*) otoliths sampled from two recognised plaice nursery grounds along the North Wales Coast between 2004-2010.

3. Using brown trout (*Salmo trutta* L.) as a model species, to examine the efficacy of otolith microchemistry to identify natal river / geographical region of origin for juvenile brown trout (*Salmo trutta*) parr in the Irish Sea region and using this freshwater baseline to try to identify marine-caught adult sea trout back to geographical region of origin (Chapter 4).