ENVIRONMENTALLY INDUCED PHYSIOLOGICAL RESPONSES THAT DETERMINE FISH SURVIVAL AND DISTRIBUTION: A REVIEW.

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INTRODUCTION

Limitation to an aqueous habitat is the most fundamental physiological constraint imposed upon fish, phrases such as 'like a fish out of water', convey our acceptance of the general unsuitability of fish for terrestrial existence. (Davenport and Sayers 1993).

The constraints that restrict fish to an aquatic habitat relate to respiration, acid-base regulation, nitrogenous excretion, water balance and ionic regulation.

A fish not adapted for an amphibious lifestyle when removed from water, becomes hypoxic and hypercapnic and soon succumbs to respiratory acidosis (Heusier, 1984), because the problem of excretion of H^+ and CO_2 are more immediate than lack of oxygen. This happen because fish gills collapse in air. while the ventilator arrangements that move an incompressible medium (water) oven them become ineffective.

AIR-BREATHING

Many fish have coolured the ability to breathe air (see Sayers and Davenport, 1991; Datta-Munshi and Hughes, 1992 for discussions). This ability apparently evolved in response to two environmental phenomena: general warming and drying up of shallow freshwater in the Devonain period, and reduced environmental oxygen tensions. some 90% lower than at present (Tappan, 1968). Under such conditions the quantity of O_2 in solution is extremely low and the advantages of using the much richer source of O_2 in air (25-40 times greater V/V than in water. depending on temperature) are particularly strong. This led to the evolution in the Tertiar period of most air-breathing teleosts groups especially in swampy habitats with inherently low aquatic O_2 tensions that were exacerbated by high organic loading.

PROBLEMS OF NITROGENOUS EXCRETION

Invasion of land also involves problems of nitrogenous excretion. Ammonia is the principal nitrogenous end-product excreted by aquatic, gilled organisms (Kormarik and Oarmeron, 1981). It is toxic, however, if present in excess. In the aquatic environment this disadvantage is offset in that its formation, during the catabolism of protein, and nucleic acids, is metabolically inexpensive, and it requires no modification prior to excretion. Although ammonia is principally excreted in the unionised form some ammonia may be eliminated as NH_4^+, the NH_4^+ being exchanged for Na^+ (Maetz 1974; Evans, 1977). Over 97% of body ammonia is present in ionised form. Following the pioneering work Smith (1929) it is assumed that 90% of nitrogenous excretion takes place at the gills, predominantly in the form of unionised ammonia. It should be noted that though this is true of many freshwater teleosts. this is not the case for marine and amphibious fish, Marine fish excrete more nitrogen via the skin and gut than freshwater fish, so that only 50-70% of nitrogen is excreted by the gills (Sayers and Davenport, 1987a, b).

In some species which leave water for appreciable periods of time, a common response to the lack of external water to carry ammonia away from the body involves a reduction in

nitrogen metabolism, a shift from ammoniotelism to ureotelism, and the accumulation of urea during the period of emersion e.g. mudskippers (Gordon et. al, 1969,1970 and 1978).

Reduced nitrogen metabolism and storage of urea (for subsequent excretion, mainly across the gills when the fish returns to water) are the standard responses of well-adapted amphibious fish to emersion, and set a time limit to such emersion. This time limit may be a matter of hours or days in the case of active teleosts, or months (years in the case of aestivating lung fish which accumulate very high levels of urea in their body fluids Smith, 1930; Janseens,1964, Forster and Goldstein, 1969).

Maintenance of water balance is a problem for all living amphibious fish when in air, Mudskippers, cling fish and others tolerate considerable water loss (as much as 20-47%. Gordon et. al. 1969, 1970; Eger 1971. Daniel, 1971 and Bridges, 1993).

Lungfish can only avoid desiccation for a period of months by aestivating in mucous cocoons within holes in (initially) wet mud. For freshwater amphibious fish, return to water will allow water balance to be restored by osmotic uptake across skin and gills; for marine species drinking will be necessary, with subsequent expenditure of energy in pumping of ions at the gut and the gills (Rankin & Davenport, 1981). Most amphibious fish do not eat or drink when out of water (there are exceptions, such as the rock skipper, *Alticus kirki* (Gunther) that browses upon algae when immersed (Brown et al (1991), so it is inevitable that ion regulation (mainly at the gills) must also be limited to the periods of immersion.

Clearly there are pressing physiological reasons why the bulk of fish species are restricted to aquatic habitats. However, there are also considerable anatomical barriers to a terrestrial, existence, mainly concerned with locomotion and skin structure. These barriers were surmounted by fish when tetrapods (Amphibian) evolved either from osteolepiform fish, or from a common ancestor with lung fishes during the late Devonian/early Carboniferous period (300-350 mybp Milner *et al.* 1986).

DEPTH OF WATER.

Photographs taken by Piccard of Tripod fish (Chlorophthalmidae) from the bathyscope in the deepest of sea—trenches, revealed that there were no lower limit to fish distribution (Piccard and Dietz, 1961). Midwater and bottom trawling, filming and submersible observations show that a diverse ichyofauna may be found at all depths, in all seas. There do appear to be constraints on the sort of fishes that occur at increasing depths. Although these may, in large measure be due to a restricted energy supply and lack of light, particularly in the pelagic domain, there are aspects of fish physiology that may reinforce these constraints.

The two major physical problems imposed by great depth are increasing pressure, and below 1000m low temperature. There is also an important chemical problem. Surface sea water is practically a saturated solution of calcium carbonate, but the solubility of this salt increases with pressure and decreasing temperature so that calcareous sediments are not found on the sea bed below a depth of around 3000m. The problems of pressure and calcium carbonate interact in the physiology of fish buoyancy. Teleost fish primarily have swim bladders and these are found in about 75% of Mesopelagic fish which live at depths down to about 1000m. At greater depth, bathypelagic fish usually have no swim bladder, or a swim bladder filled with fat, and they show a progressively reduced musculature and ossification. Generally, the lack of ossification has been interpreted as an adaptive feature, aiding buoyancy. However, a reduction in hard, calcareous tissue may be an energy-saving strategy in an environment where food is sparse and dissolution of calcium carbonate takes place. Marshall (1979) also notes that benthopelagic fish (i.e. those interacting with the sea bed) retain swim bladders even when distributed to depths of as much as 7000m. They are also much more robustly constructed than benthopelagic fish, and have substantial well-ossified skeletons. It is now known that benthopelagic fish have a much richer food supply than bathy pelagic fish (Gage and Tyler, 1991). Given this energy supply, it may be

suggested that they can 'afford' the energetic costs associated with swim bladder function. the alternative buoyancy mechanism seen in some species (Stein and Percy. 1982) and the efficient skeleto-muscular system that facilitates their foraging

TEMPERATURE

For fish, a wealth of literature demonstrates the importance of temperature to virtually all aspects of fish biology. at all stages of life history (e.g. Brett 1970) and laxter 1992) for a recent review of the effects of temperature on fish larvae. Fish are almost allectothermic animals, even those groups that exhibit partial eacthermy have muscle/gut temperatures related to external temperatures (Carey et al. 1982). Fish are found in all aquatic environments from the cold Southern (ocean to tropical lagoons and freshwater streams affected by geothermal heat

Van't Hoff (1884) observed that the rate of chemical reactions tend to double for each . $10°C$ rise in temperature. Since the rates of individual biochemical reactions are equally affected by temperature. physiologists soon introduced the concept of Q_{10} relationship for consideration of metabolic rate.

$Q_{10} = (R_1/R_2)$ $(^{10}/t_1-t_2)$ where R_1 is the metabolic rate at temperature T_1, and R_2 is the metabolic rate at temperature T_2.

A Q_{10} of 2 indicates that the metabolic rate behaves as predicted from Vant's Hoff's simple chemical findings. Krogh (1916) established long as that intemperate fish Q10tended to be high (10.9) at low temperatures (0-5°C) and approached 2 only at temperatures around 30°C. Such values indicate that fish are unduly limited in their rates of living at low temperature, being even more sluggish than consideration of simple rates of chemical reactions would permit (Davenport and Sayers 1993) experiments have even shown at least partial (Pretch type 3, Pretch. 1958) compensation over surprisingly wide temperature ranges within a single species. Kent et al (1988) studied the channel catfish *lictalurus punctatus* (Rafinesque). demonstration pretch type 3 response (Q10 < 2) between 0°C and 30°C when moved from high (25°C) to a lower temperature (15°C) environment, catfish showed profound anatomical, histological and biochemical changes to the liver (a doubling of the liver mass, cell size, total protein and total enzyme activity) together with cardiac hypertrophy. all features suggesting an increased cost of maintenance at low temperature. It is currently impossible to determine how much of the relatively high overall metabolic rate at low temperature is due to enhanced maintenance costs. so that no estimate of the scale of benefit of this strategy can be made.

Hochachka (1988) and Dunn (1988) argued that life style as well as length of evolutionary history must also be considered in evaluation of metabolic compensation. Antartic fish (Predominantly benthic or semi pelagic) living in productive inshore waters at about − 1.9°C do tend to have relatively high metabolic rate (See Montgomery and Wells, 1993 for review) well within the ranges exhibited by temperate species. Bathy pelagic fish, living at equally low temperatures. but at great depth have far lower metabolic rate (by as much as one or two orders of magnitude), presumably because they are adapted to an environment in which food supply is low and unpredictable. It is tempting to accept that the inherent complexity of ecological systems makes it difficult to discern fundamental physiological limitations deriving from environmental temperature. Hochachka also postulates that Antartic fish have evolved the metabolically expensive route of increased pump densities, and so have a high metabolic rate, while deep water fish have evolved reduced permeabilities, so can maintain the low metabolic rate that is favourable in their cold and energy-poor environment. Hochachka presents some debatable evidence in favour of his hypothesis (see Davenport. 1992; Davenport and Sayers 1993 for critique). But. the basic premise offers the promise of explanations of why temperature may physiologically restrict the sort of fish that live in a particular environment. If the osmotic physiology of fish becomes fundamentally more difficult and expensive at low temperature

(demanding more compromises at the cellular and membrane level than at higher temperatures), then certain sorts of lifestyle become less tenable.

High Temperature as a distributional constraint has been attracting relatively more attention in recent time, though very little work has been done in the tropics in this regard. Antarctic fish soon die if exposed to temperatures much above +5°C (Montgomery and Wells, 1993). Fry et. al. (1942) predicted a lethal temperature of 30°C for the majority of temperature fish, and these is plenty of anecdotal evidence of fish avoiding warmed surface waters in high summer. Thermal pollution by power stations has certainly resulted in fish kills (e.g. Gallaway and Strawn, 1974). Laboratory experiments have also demonstrated the abilities of fish to move along thermal gradients and to choose thermal optima (see Davenport, 1985 for review). However, in an absolute evolutionary sense, it would seem that fish are no more physiologically constrained by elevated temperatures than other aquatic animals, most of which are incapable of tolerating temperature s higher than 45°C. It is also indeed possible to argue that fish generally benefit from high temperatures (within their basic biochemical limits of survival), since these are associated with high levels of activity, rapid food processing and digestion. However, high temperature, high activity life styles are, only sustainable if abundant food is available (See Jobling, 1993; Davenport and Sayers 1993 for review), so there are additional indirect physiological constraints operating in warm environments.

OXYGEN TENSION

Fish are generally intolerant of substantially reduced oxygen tensions. Davis (1970) reviewed information for a wide range of freshwater, marine and migratory fish. A number of species (particularly anadromous species) show immediate signs of disturbed physiology (usually expressed initially in heightened activity and ventilation rate) if their environment is less than fully air-saturated. Scope for activity is also reduced in such fish if their environment is oxygen-depleted, while Eddy (1981) reported disturbances of osmotic and ionic physiology in freshwater and marine fish exposed to hypoxia. Avoidance of O_2 depleted water is also common in such sensitive species, so in that sense physiology determines distribution. Another effective behaviour involves aquatic surface breathing (ASR) in which fish respond to extremely low levels of O_2 by exploiting the surface film where diffusion maintains significant dissolved oxygen levels (Kramer, 1987). Numerous freshwater species and a few marine species are bimodal capable of breathing air as well as extracting O_2 from water; they show enhanced frequencies of air-breathing when exposed to hypoxic water calculations demonstrate that the energetic costs of air-breathing (including locomotors transport to and from the water surface) are below those of water-breathing under hypoxic conditions (Bergen and Kramer, 1987).

Most freshwater and marine ecosystems have high oxygen tension because of turbulent oxygen exchange with the atmosphere, plus photosynthetic activity in the emphatic zone. Consequently O_2 availability poses little physiological problem for fish in the bulk of the aquatic environment. However, the limitations of fish respiratory physiology are revealed by localized eutrophication resulting from man's activities. Fish kills in freshwater systems, following wet season run-off or release of sewage/agricultural slurries, still often provide the first indication of pollution incident. Generally, such incidents cause anoxic conditions, and fish probably die because they have limited anaerotic capacity, although sulphide toxicity may be a factor in some cases.

In coastal waters too, there are now signs of episodic oxygen depletion, perhaps mainly because of agricultural run-off, and such events are often associated with algal blooms. As with river systems complete O_2 depletion has been recorded, resulting in massive fish kills, which are referred to as natural fish kills. Fish responses to complete anoxic conditions are generally ineffective, though a few apart from catfishes can survive anoxic for minutes even, a few hours. A few species, notably goldfish (C. auratus) and carp (Cyprinus carpio) respond by reduced

activity and detoxification of lactate accumulating from glycosis by the synthesis and excretion of ethanol (Johnston and Bernard, 1983).

Those fish that live in environments featuring episodic oxygen depletion either avoid the affected area, respond by reduced activity, or die. Resident fish from such environments are benthic and intermittently active, so respiratory physiology tends to exclude active pelagic fish from hypoxic areas. However, a few active fish do exploit areas subject to hypoxia (Pihl et. al.1992).

Fish are probably all (oxygen) oxyregulations (Nonotte et al., 1993) and rely on haemoglobins for oxygen transport. Weber (1990) reports that fish to an extent greater than other vertebrates possess multiple haemoglobins. Multiplicity of haemoglobins has many effects and advantage. But heterogeneity makes possible a division of labour between individual components, which extends the range of conditions under which the composite pigment executes its O_2 transporting and other functions in VIVO, enlarging the inhabitable environment. Fish from environments with variable oxygen availability show particular heterogeneity of haemoglobins (an average of four different Hb component; Kunzmann et. al, 1992) enabling them to load and unload oxygen efficiently over a wide range of oxygen tension.

Rockpools can feature hyperoxic environments, with O_2 tensions rising to 300% air saturation because of photosynthetic activity (Bridges et al, 1984). Some freshwater habitats can become supersaturated too. Relatively little study has been made of the effects of hyperoxia, but available evidence for both rock pool fish and freshwater species suggests that metabolism is unaffected, fish responding by reduced gill ventilation. There is no hint that hyperoxia poses stressful problems or limits distribution.

SALINITY.

Many species of fish are stenohaline, either in freshwater or in the sea. Others are euryhaline. As a result of this fish live in a wide variety of salinities, ranging from freshwater to hyper saline lagoons and pools. In general, the upper salinity limit for fish is about 85-90% (Rankin and Davenport, 1981). The osmoregulatory physiology for fish does seem to limit distribution directly, since a number of invertebrates; particularly crustaceans (e.g. Artemia, Trigriopus) can survive much higher salinities (>300%)

Elasmobranch fish, physiologically adapted to seawater by virtue of a high blood urea level, and a urea-tolerant biochemistry, are predominantly limited to the marine habitat (Griffith and Pang, 1979). However, there are sufficient species of elasmobranchs living in brackish or freshwater (e.g. sawfishes, pristidae, river stingrays, potamotrygonidae) (Bone and Marshall. 1982) for it to be unlikely that a fundamental physiology constraint is present.

Some euryhaline fish can adapt to salinity changes over periods of days (e.g. brook charr, *Salvelinus fontinalis* Mitchell; Castonguay et. al. 1982; Pelletier and Besner, 1992), others can cope with abrupt changes in a matter of minutes (e.g. Shanny; House, 1963), and it is in this respect that there are generally physiological constraints on fish that are imposed by salinity.

Most freshwater fish die if placed in seawater, because they cannot pump salts outwards, so succumb to an osmptic outflow of water and inward diffusion of salts. Likewise a marine fish held in freshwater dies because if cannot pump salts inward across the gills to maintain plasma osmolarity. Such mortality is almost immediate (Amousiq. 1972). Euryhaline fish, therefore have to be able to pump salts in both directions, and have to be able to control integumentary permeability. However, those fish that are capable of withstanding sudden and frequent salinity changes are generally specialized morphologically and physiologically in ways that restrict their life style. Most of such highly euryhaline fish are small and have thickened gills with a relatively low surface area, and a low integument permeability to salts and water. These features slow salt and water movements, reducing the cost of ion regulation (Davenport and Vahl, 1979).

A few teleosts migrate between the extremes of seawater and freshwater, yet do not exhibit small size and low energy life style. In salmonids, such migration involves considerable expenditure of locomotory energy, increased osmorregulatory costs and increased risk of predation, apparently offset by the enhanced feeding opportunities while at sea (Gross et.al, 1988, Feltham, 1990). Physiological adaptation involves changes in skin permeability, alterations in chloride cell numbers, redistribution of tissue water, changes in plasma chloride concentration and alterations in gill Na+, K+ ATPase activity (e.g. Houston 1964, Boeuf, 1987; Salman and Eddy, 1987; Pelletier and Besner, 1992).

The ionic content of freshwaters is determined by the chemistry of the rocks and soils over and through which they flow before reaching streams, rivers or lakes. Soft waters can almost equal distilled water in their ionic content, with dissolved calcium in some areas as low as 1-3 mmol^{-1} (Sayers et. al, 1993). Hard waters typically have calcium concentrations of 670-1790 mmol^{-1} (Reader et. al. 1991). When external calcium concentrations are low, calcium ions, which are vital to the integrity of membranes and to control of their permabilities may be lost at their gills (McWilliams, 1983). In consequence, few fish species can survive in waters with conductivities below 10m^S cm^{-1} (calcium concentrations < 10 mmol 1^{-1}), probably because their gills become too permeable and osmoregulation fails.

pH

Fish possess limited internal buffering capacity and are therefore handicapped in their control of internal pH which is in the range of 7.4-8.4. This is coupled with the fundamental limitations of ventilatory control of pH imposed by the viscosity of water (Heiseler, 1984). This therefore means that acid-base balance is much more a matter of excretory rather than respiratory control.

In freshwater, pH levels are naturally far more variable than in the marine habitat. Most marine fish live in a slightly alkaline environment (pH 7.8-8.0) that has an enormous buffering capacity for H+ and CO_2. pH therefore poses no problem for them.

Rockpool fish do encounter pH changes associated with the diurnal alternation of photosynthetic and respiratory dominance. The extreme values reported are low and around pH 7.2-7.3 at night when the pool biota produces much CO_2, and highs of 10.0 to 10.2 when photosynthetic activity is at maximum and PCO_2 reduced (Ganning, 1971; Truchot and Duhamel-Jouve, 1980; Morris and Taylor 1983). And there is no evidence of any significant constraint imposed by these values.

There is now abundant evidence that acidification, particularly of soft water is damaging to fish, and that body of soft water that feature (naturally or artificially) episodic or chronic pH levels of about 4.0-4.2 or less are devoid of fish. Stress and death due to acidification are not simple physiologically, because acidification is invariably associated with waters of low conductivity (e.g. low calcium content) and elevated trace metal levels. Although acidified water may impair physiological processes, including gas transfer, ionic regulation and acid-base balance (McDonald, 1983; wood 1989; Howells 1990; Sayers et al. 1993). The available information on acid-stressed fish, suggests that survival at a low pH demands considerable (and presumably energy-expensive) physiological adaptation that will compromise physiological capacity in other directions. More data are clearly needed to assess physiological costs. Exposure of freshwater species to alkaline water (pH 9.5) inhibits ammonia excretion (Wright and Wood, 1985; Wilkie and wood, 1991; Wilkie et. al, 1993), although this inhibition is more dramatic in alkaline soft water than alkaline hard water (Yesaki and Iwama, 1992). Clearly in water of pH 9-10 the excretion of ammonia is a physiological challenge, and fish may need to utilize special mechanisms to maintain nitrogen excretion in severely alkaline environments (e.g. Wright et. al. 1993).

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