The endocrinology of stress in fish: an environmental perspective

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ABSTRACT

Much of the understanding of the endocrine basis of stress in fish comes from studies of cultured stocks of teleost; there is comparatively little information on stress responses in wild stock, and less still on chondrosteans and elasmobranchs. This understanding is being refined through increasing understanding of molecular processes underlying endocrine events, with molecular tools offering ready examination of parts of the endocrine pathway that have been resistant to easy measurement of hormone products. An assessment of the timecourse of activation of the hypothalamic-pituitary-interrenal axis shows generally strong independence of temperature, with most teleosts showing measurable increase in plasma cortisol within 10 mins of stress. Chondrosteans and elasmobranch responses are less well described, but in chondrosteans at least, the response pattern appears to be similar to teleosts. The short latency for increases in corticosteroids following exposure to a stressor means that sampling of wild fish needs to occur rapidly after encounter. Several techniques including underwater sampling and rapid line capture are suitable for this, as is measurement of steroid release to the water by undisturbed fish, albeit possibly with a reduced range of applications. Basal cortisol values in wild teleosts are typically $< 10 \text{ ng mL}^{-1}$, but a number of species show values orders of magnitude higher in unstressed fish. Variability in corticosteroid levels arises from a range of factors in addition to stress including, sex and maturity, time of day or since feeding, and season. These factors need to be understood for the sensible assessment of stress responses in wild fish. Studies on free-living birds suggest that environmental stress resides mainly around unpredictable change, and the limited data available for fish support this view. The effect of unpredictable event such as floods or storms are difficult to assess in wild fish due to the difficulty in sampling at these times, and would be predicted to impose environmental stress as in terrestrial systems; however, this has yet to be demonstrated. There is scope for use of stress responses to be used as a measure of environmental quality but only if the basic response to environmental stress is well understood first. Development of this understanding remains a priority for this field of research.

Keywords: teleosts, chondrosteans, elasmobranchs, corticosteroids, cortisol, environmental stress

1. Introduction

There is now extensive literature on the physiological and endocrine basis of stress in fish, largely constructed from studies of captive or cultured fish, and within this, largely examining the effects on teleosts fishes (see reviews by Barton and Iwama, 1991; Pickering, 1998; Sumpter, 1997; Wendelaar Bonga, 1997; Barton, 2002). Because of the potential difficulties associated with accessing and suitably sampling fish in their natural environment there is considerably less information on stress and its physiological and endocrine effects, in natural settings (eg. Pankhurst and

Sharples, 1992; Carragher and Rees, 1994; Pankhurst and Dedual, 1994; Morgan and Iwama, 1997; Grutter and Pankhurst, 2000; Pankhurst, 2001; Frisch and Anderson, 2005). Again, because of the limitations imposed by access to natural populations and also until relatively recently, the limited interest in the population status of many non-teleost species, there is very little information on stress processes in non-teleost groups (Barton, 2002). The subset of investigations of non-teleosts in the natural environment is smaller still.

The study of stress in natural populations is confounded by the generally high sensitivity of wild fishes to disturbance, and the fact that this disturbance in turn can change many of the endocrine or physiological variables of interest. This problem is not unique to studies of wild fishes but is exacerbated by the constraints imposed by capture and sampling requirements for field studies. This means that establishing credible resting or baseline data for unstressed fish in the wild is of critical importance, as is an understanding of how the same endocrine variables might change in relation to processes where the primary driver may not be the response to a stressor.

The above considerations in turn raise some more general questions, one of which is the degree to which fish in the natural environment actually experience stress in the way it has usually been induced, in studies of captive fish. For example, the capture and confinement regimes typically used to elicit stress in laboratory situations might only equate in the wild to extreme events such as pursuit and capture by a predator, where the outcomes are more proximate and stress responses of the type seen in the laboratory may in consequence, be quite rare. If this is the case, then the occurrence of detectable or sustained stress responses in wild fish are more likely to be indicative of broader environmental challenge or change. This effect has been well described in birds where unpredictable environmental changes such as extreme weather or storms initiate stress-mediated changes in behaviour (reviewed in Wingfield, 1994; Wingfield and Ramenofsky, 1999; Cyr and Romero, 2009). There is a caveat to this observation in that the ability to detect such stress responses rests strongly on the ability to distinguish stress-mediated effects from normal changes in physiology associated with behaviour, nutritional status and life history events (Busch et al., 2010).

The occurrence of severe and, or sustained stress responses among individuals in natural populations is likely to have life history consequences through the generally inhibitory or interruptive effects of stress on growth and reproduction (Pankhurst and Van Der Kraak, 1997; Pottinger, 1999; Milla et al., 2009; Schreck, 2010), behaviour (Schreck et al., 1997; Wingfield and Ramenofsky, 1999) and disease resistance (Balm, 1997; Maule and VanderKooi, 1999). It is unclear how common these effects are in the natural environment but a reasonable prediction is that environmental change that induces a significant stress response among individuals of a population, will if sustained, also have impacts at the population level.

The intent of the present review is to summarise the current state of knowledge of the endocrinology of stress in teleosts with reference to earlier comprehensive reviews on the subject, and to examine the level to which these processes might operate among non-teleosts, to the extent that current understanding allows. This is followed by a consideration of the understanding of stress processes as they occur in natural populations of fish, and how they might change in response to environmental change or challenge. Finally, there is consideration of the usefulness of stress physiology as a predictive or diagnostic tool for the assessment of the status of wild populations, or as sentinels of environmental change.

2. The stress response

2.1 Teleosts

The stress response in teleosts is biphasic with an initial short latency increase in plasma levels of catecholamines released from stores in the chromaffin tissue of the kidney, followed by a longer latency but usually more prolonged elevation in plasma cortisol levels following *de novo* synthesis by interrenal tissue. Description of both responses has been made in detail elsewhere (eg. Sumpter, 1997; Wendelaar Bonga, 1997; Pickering, 1998) and will only be summarised here with focus on aspects of particular relevance to environmental aspects of the stress response. Rapid increases in plasma levels of the catecholamines, adrenaline and noradrenaline occur mainly in the face of severe acute stress, particularly if that stress is accompanied by, or involves significant reductions in blood oxygen content. This rapid response is primarily mediated by cholinergic nerve fibres that directly innervate the chromaffin tissue; however, there is also evidence for involvement of serotonin, adrenocorticotrophic hormone (ACTH), angiotensin II and non-cholinergic innervation of chromaffin tissue in modulation of catecholamine release (reviewed in Perry and Bernier, 1999). Effects of increased plasma levels of catecholamines include increased haemoglobin oxygen affinity, in some species increased arterial blood pressure (Perry and Bernier, 1999) and in most species examined, release of stored hepatic glycogen to the plasma as glucose (Wendelaar Bonga, 1997). The rapid nature of the increase in plasma catecholamine levels post-stress makes measurement of the timecourse of the response problematic using standard sampling techniques (Sumpter, 1997). This can be addressed by serial sampling from fish implanted with aortic cannulae, where stress-induced increases in plasma catecholamines occur within a minute of disturbance (Lowe and Wells, 1996). However, cannulation also typically stimulates a chronic increase in plasma cortisol levels (Gamperl et al., 1994; Lowe and Wells, 1996; Cech et al., 1996) making separation of effects due to acute stress from those generated by the chronic stress of cannulation potentially difficult. This concern may be ameliorated to an extent by the conclusion of Perry and Bernier (1999) that there is not compelling evidence for any effects of low level or moderate stress on catecholamine levels.

Activation of the corticosteroid limb of the stress response occurs via the stimulation of the hypothalamo-pituitary-interrenal (HPI) axis culminating in the release of cortisol to the circulation (Sumpter, 1997; Wendelaar Bonga, 1997; Pickering, 1998). Stress stimulates the expression and synthesis of corticotropin releasing factor (CRF), with CRF transcripts being concentrated in the preoptic area of the brains of rainbow trout *Oncorhynchus mykiss* (Ando et al., 1999; Doyon et al., 2003, 2005, 2006; Bernier and Craig, 2005; Craig et al., 2005), masou salmon *O. masou* (Westring et al., 2008), carp *Cyprinus carpio* (Flik et al., 2006) and the preoptic area and telecephalon of goldfish *Carassius auratus* (Bernier et al., 1999). In mammals this process is regulated by transcription factors activated by the cAMP-dependent protein kinase A pathway (Yao and Denver, 2007). CRF expression is in turn subject to negative feedback by cortisol (Doyon et al., 2006; Bernier et al., 1999). Studies on other vertebrate groups have demonstrated further complexity in the modulation of CRF expression with corticosteroids in mammals having inhibitory effects on CRF transcription at high concentrations, but exerting permissive action on the stress activation of CRF expression at lower levels (Yao and Denver, 2007). Corticosteroids typically exert their actions through classical genomic action but in addition there is evidence for rapid non-genomic action via G-protein coupled membrane receptors in mammals (Tasker et al., 2006) and also fish (Borski et al.,

2001). CRF neurons project to the proximal pars distalis where CRF release occurs in close proximity to corticotrophs (Lovejoy and Balment, 1999) where it stimulates the synthesis and cleavage of the precursor pro-opiomelanocortin to ACTH, and under severe stress β -endorphin and α -melanocyte stimulating hormone (α -MSH) from the intermediate lobe of the pituitary (Sumpter, 1997). The limited data available for fish show that ACTH concentrations rise in the plasma of coho salmon *Oncorhynchus kisutch* and rainbow trout within a few minutes of stress (Sumpter et al., 1986). Generalisation to other species requires additional information especially given the initial lack of detectable effect of acute stress (where there was a rapid increase in plasma cortisol levels) on plasma ACTH levels in tilapia *Oreochromis mossambicus* (Balm et al., 1994). ACTH levels did rise after 3 h confinement in behaviourally subordinate tilapia, and also confined fish in a later study on rainbow trout (Balm and Pottinger, 1995).

In addition to stimulating ACTH release, CRF also has neuromodulatory and behavioural effects. CRF has been demonstrated to suppress appetite and feeding behaviour in a range of species (Bernier and Peter, 2001; Bernier and Craig, 2005; Bernier, 2006) and also stimulates increases in general locomotor activity (Clements et al., 2002; Lowry and Moore, 2006), and downstream movement in salmonids (Clements and Schreck, 2004). This suggests that at least some of the behavioural effects occurring in stressed fish (Schreck et al., 1997) may arise proximally from the direct action of CRF, rather than as downstream effects of activation of the HPI axis.

The timecourse for the interrenal response to ACTH by the production of cortisol is variable among species but typically has a latency of minutes rather than hours. Examination of the responses latency (defined as the time to detect statistically significant elevations of plasma cortisol) in a range of species where fish had credible basal levels and where sampling was conducted over a suitably short timeframe to allow detection of early increases in cortisol, showed response times from as short as 2.5 min in striped bass *Morone saxatilis* (Tamasso et al., 1996) to as long as 120 mins in the sea raven *Hemitripterus americanus* (Vijayan and Moon, 1994) (Table 1). With the exception of the Antarctic species *Pagothenia borchgrevinki* where residence at -1.9°C is correlated with a response latency of ~ 60 mins (Ryan, 1995), there is not a strong correlation between temperature, and the rate at which fish respond to stress with an increase in plasma corticosteroid concentration (Table 1). Fish at holding temperatures of $<10^{\circ}\text{C}$ showed response latencies as short, or shorter than species held at up to 30°C . Instead, it appears that response latencies relate to lifestyle differences, with the suggestion that more sedentary species show reduced or slower post-stress increases in cortisol (Vijayan and Moon, 1994; Wright et al., 2007). Excluding the sea raven and Antarctic examples, the mean response latency recorded across all species and temperatures listed in Table 1 is 12.5 mins, and this is consistent with the presumed adaptive value of being able to mount a corticosteroid response to a stressor over an appropriately short timeframe (Wendelaar Bonga, 1997; Mommsen et al., 1999).

There is considerable variability in the magnitude of the corticosteroid response among species, with peak values typically being in the range of $30\text{--}300\text{ ng mL}^{-1}$ but also varying within species according to the duration or severity of the stressor (reviewed in Barton, 2002). Recovery from stress in terms of the return of plasma cortisol to resting levels appears to occur over periods of hours rather than minutes, but assessment of this is confounded by the fact that measurement usually involves subsequent maintenance and manipulation of fish under conditions that may themselves be perceived as stressful. This means that rates of decline may be slowed

if stressors still exist in the recovery environment. More rapid falls in plasma cortisol may reflect recovery but they can also arise from desensitisation of the HPI axis resulting from sustained exposure to stressors (Cyr and Romero, 2009). With these caveats, recovery from exposure to acute stress has been reported to be as short as 2-6 h (Pickering et al., 1982; Robertson et al., 1988; Young and Cech, 1993) or as long as 24-48 h (Pankhurst and Sharples, 1992; Vijayan and Moon, 1994; Barnett and Pankhurst, 1998). More definitive assessment of recovery profiles might ideally involve release of stressed fish back to the natural environment with single recapture and sampling at intervals during recovery. This is difficult to achieve in the natural setting but is approached by the use of artificial stream systems for measuring recovery in salmonids. Experiments with wild brown trout *Salmo trutta* show return to capture levels of cortisol within 6 h (Flodmark et al., 2002) and in a later study at the earliest post-stress sample time of 24 h (Arnekleiv et al., 2004). Wild migratory rainbow trout *Oncorhynchus mykiss* held in cages in the river after line capture showed recovery of plasma cortisol within 24 h (Pankhurst and Dedual, 1994). This suggests that under most circumstances, recovery from acute stress will occur in most species over a period of about 6 h, and longer recovery periods may be artefacts of the holding conditions and sampling regimes.

In addition to increases in plasma cortisol, stress typically causes increases in plasma glucose and lactate levels. Elevations in plasma glucose are generated initially by catecholamine-mediated glycogenolysis and at later stages, cortisol-mediated gluconeogenesis, and lactate concentrations rise as muscle lactate formed during anaerobiosis is released to the plasma (reviewed in Begg and Pankhurst, 2004; Mommen et al., 1999). Post-stress increases in plasma glucose and lactate are sometimes used as proxy measurements for activation of the HPI axis (and see later discussion on elasmobranchs) but this approach may need to be used with caution. Rises in plasma glucose may be limited in species with limited hepatic glycogen stores (eg. Pottinger et al., 2002; Wright et al., 2007) or show different increase and recovery profiles from cortisol (Pickering et al., 1982, Pottinger, 1998; Flodmark et al., 2002). Protocols separating out confinement and exercise components of stressors show that plasma cortisol levels in salmonids can increase without any change in plasma lactate, and *vice versa* (Thomas et al., 1999). Other groups such as flatfishes, syngnathids and tropical labrids may undergo *in situ* recycling of muscle lactate with limited release to the plasma, despite stress-induced elevations in plasma cortisol (Barnett and Pankhurst, Grutter and Pankhurst, 2000; Wright et al., 2007).

Increases in plasma corticosteroids have a wide range of metabolic effects including as noted above, the modulation of carbohydrate metabolism through gluconeogenesis, increases in protein turnover, regulation of amino acid metabolism, ammonia output, glutamine synthetase and aminotransferase activity, and increased lipolysis (reviewed in Mommsen et al., 1999). Cortisol plays a key role in osmoregulation largely through its effect in stimulating gill Na^+/K^+ -ATPase activity (reviewed in McCormick, 1995) at lower levels, probably has permissive effects on female reproductive events during the peri-ovulatory period (Milla et al., 2009), modulates the tissue inflammatory response through inhibitory effects on cytokine production (reviewed in Aluru and Vijayan, 2009) and a range of other immune system responses (Maule and VanDerKooi, 1999), and appears to attenuate the cellular heat shock protein response to thermal insult (Ackerman et al., 2000; Basu et al., 2001). The collective effect of these actions is for cortisol to act as a regulatory factor for a wide range of physiological functions under normal conditions, and also to allow for rapid physiological readjustments in the face of exposure to stressors

(Mommensen et al., 1999). There can be a tendency for interpretation of these effects as being largely inhibitory on processes such as growth, reproduction and disease resistance but this perception largely comes from understanding of the effects of stress in culture situations (reviewed in Pickering, 1998) where there is little scope for activation of the stress response to actually make a difference to the situation that gave rise to the response. The key point is that the stress response is fundamentally a regulatory response to return the animal to 'homeostatic norms' (Schreck, 2010).

2.2 Chondrosteans

There is relatively little information on the stress responses of non-teleost osteichthyan species, with most information derived from studies on sturgeons, stimulated either by interest in aquaculture or preservation of endangered natural stocks. In general, the corticosteroid response to a range of stressors appears to be of similar pattern to that found in teleosts but with both low baseline values and modest subsequent increases in plasma cortisol reported from a number of studies of captive or cultured stocks. The pallid sturgeon *Scaphirhynchus albus* had resting cortisol concentrations of ~ 0.7 ng mL⁻¹ and these increased to 10.7 ng mL⁻¹ after 6 h of 'severe' confinement stress. GCMA analysis of plasma confirmed that cortisol was the main corticosteroid present (Webb et al., 2007). Glucose concentrations increased with sampling in both control and stressed fish, but plasma lactate remained low and unchanging in both groups. Barton et al. (2000) reported slightly higher resting levels (2-3 ng mL⁻¹) for the same species and similarly modest elevations following handling and confinement. Juvenile great sturgeon *Huso huso* showed a moderate increase in plasma cortisol (9-19 ng mL⁻¹) in response to air exposure and this was accompanied by a short term peak in plasma lactate and a small but more sustained increase in plasma glucose (Falahatkar et al., 2009). Similarly modest increases in plasma cortisol following stress have been reported in cultured Atlantic sturgeon *Acipenser oxyrinchus* (Baker et al., 2005), and white sturgeon *A. transmontanus* (Belanger et al., 2001; Zuccarelli et al., 2008). Greater increases in post-stress cortisol (to ~ 120 ng mL⁻¹) were recorded from shortnose sturgeon *A. brevirostrum* (Baker et al., 2005). Juvenile cultured paddlefish *Polyodon spathula* showed increases in plasma cortisol from 2.2 to only 11-14 ng mL⁻¹ following air exposure and chasing, although severe confinement for 6 h did elevate levels to 74 ng mL⁻¹ (Barton et al., 1998). These authors also confirmed (by HPLC) cortisol as the only measurable corticosteroid. Cataldi et al. (1998) reported variable cortisol levels in cultured Adriatic sturgeon (*A. naccarii*) (12-39 ng mL⁻¹) but no consistent change in response to imposition of crowding and handling stress. Green sturgeon *A. medirostris* also had a small cortisol response to air exposure; however, there was a diel component to the response with a greater increase in cortisol post-stress during the scotophase (Lankford et al., 2003).

The collective assessment from the above studies has been that the corticosteroid pattern of response to stress in chondrosteans is similar to that found in teleosts but with generally smaller magnitude and possibly slower responses, and some suggestion that this represents lower susceptibility to stress. Examination of data from the limited number of studies that have sampled wild adults suggests that this may not be the complete story. Migratory adult stellate sturgeon *A. stellatus* and Russian sturgeon *A. gueldenstadtii* captured from the Volga River had post-capture cortisol levels of 67-98 and ~ 30 ng mL⁻¹, respectively. These rose to 242 and 294 ng mL⁻¹ in female and male stellate sturgeon after 9 h of holding, and to ~ 140 ng mL⁻¹ in Russian sturgeon after 10 days in holding tanks (Bayunova et al., 2002). Semenkova et al. (1999) reported similar elevations at capture in stellate, Russian and giant sturgeon and suggested that the high cortisol levels recorded may have reflected the capture of

fish during anadromous migration. The finding of both high cortisol levels at capture, and further increases subsequent to exposure to husbandry stressors suggests that the low levels recorded from other studies of hatchery stocks might reflect the effects of domestication, an element of habituation or desensitisation, or the effect of life history stage. This further suggests that interpretations of patterns of response to stress in wild fish should only be extrapolated from studies of cultured juvenile or immature fish, with considerable caution.

2.3 Elasmobranchs

Elasmobranch stress physiology differs from that of teleosts and chondrosteans in that the major circulating corticosteroid is not cortisol. The presence of 1α -hydroxycorticosterone (1α -OHB) as the main plasma corticosteroid has been confirmed in *Raja* spp. (Idler and Truscott, 1966, 1968, 1969; Kime, 1977), the dogfish *Scyliorhinus canicula* (Hazon and Henderson, 1985) and *S. caniculus* (Kime, 1977). *In vitro* incubation has also demonstrated the capacity of interrenal tissue to convert corticosterone to 1α -OHB in the rays *Raja laevis*, *R. clavata*, *R. erinacea*, and *Dasyatis violacea*; the dogfish *Squalus acanthias* and *Scyliorhinus stellaris*; and mako *Isurus oxyrinchus*, sickle *Carcharhinus falciformis*, dusky *C. obscurus*, blue *Prionace glauca* and hammerhead *Sphyrna lewini* sharks (Truscott and Idler, 1968). A systematic investigation of response to stressors, timecourse and magnitude of the corticosteroid response has not been performed in any of these species; however, basal levels appear to be similar to those reported for cortisol in teleosts and chondrosteans (Table 2). The sampling regimes were not the primary focus of these studies so it is not clear the extent to which values at the higher end of the range (139 ng mL^{-1}) reflect a response to stress.

More thorough investigation of the stress responses of elasmobranchs is limited by the current non-availability of sources of 1α -OHB suitable for use as a reference steroid and antigen for development of assay systems. Several studies have examined the usefulness of the more easily quantifiable corticosterone as a proxy for 1α -OHB. Rasmussen and Gruber (1990) measured plasma corticosterone levels in 6 species of carcharhinoid sharks but noted a lack of parallelism in the corticosterone assay which was attributed to cross-reaction with 1α -OHB in the plasma. A similar effect was reported by Manire et al. (2007) in the bonnethead shark *Sphyrna tiburo* and the Atlantic stingray *Dasyatis sabina*, with lack of parallelism in the corticosterone assay, and no measurable effect of short term confinement on putative plasma corticosterone levels. These authors concluded that corticosterone was not a good proxy for stress in these species. More recent assessments of stress have focussed on acid-base disturbance as a measure of metabolic stress. Stress associated with longline capture in 5 species of shark (dusky, tiger *Galacardo cuvier*, sandbar *C. plumbeus*, Atlantic sharpnose *Rhizoprionodon terraenovae* and blacktip *C. limbatus*) showed an inverse relationship between degree of acidosis and lactosis, and subsequent mortality (Mandelman and Skomel, 2009). This suggested that acid-base disturbance offered at least one avenue for estimating the degree of metabolic stress, and the subsequent possible fate of animals post-release.

3. Establishment of baseline values in natural populations

As discussed in the previous section, the generally short latency for increases in plasma corticosteroids following exposure to a stressor means that blood samples need to be collected as quickly as possible after first contact with capture equipment. This can be achieved by angling provided that retrieval and sampling are rapid (eg.

Safford and Thomas, 1987; Pankhurst et al., 1992; Carragher and Rees, 1994; Pankhurst and Dedual, 1994; Haddy and Pankhurst, 1998; Frisch and Anderson, 2005), but does carry the complication that exercise-induced changes in plasma metabolites are also likely to occur. A more specialised approach involves the capture of fish underwater by snorkellers or SCUBA divers, preferably with underwater sampling immediately at capture (Pankhurst and Sharples, 1992; Barnett and Pankhurst, 1998; Grutter and Pankhurst, 2000; Pankhurst, 2001). This method is only practically applicable to a subset of field studies but possibly provides the least ambiguous description of endocrine states in terms of the effects of capture and sampling on target variables. It also has the advantage of allowing correlation with immediate pre-capture behaviour with endocrine condition (Pankhurst 1990; Pankhurst et al. 1999; 2008a).

Another sampling approach that might be predicted to achieve a similar outcome is electro-fishing where there is typically a short delay between shocking and the opportunity for sampling. However, there appears to be a consistent stress effect of electro-fishing in that fish from a wide range of species show plasma cortisol levels that are elevated well over baselines established using other techniques (Schreck et al., 1976; Davis and Parker, 1986; Harrell and Moline, 1992; Barton and Grosh, 1996; Barton and Dwyer, 1997). The physiological basis for the rapid elevation of cortisol is not clear but it is accompanied by similarly rapid elevations in other parameters including plasma lactate and glucose (Bracewell et al., 2004). This suggests that electro-fishing is not a suitable capture technique for the examination of stress parameters in wild fish.

Other approaches for rapid capture and sampling of wild fish need to be considered for fish that are of small body size, either as juveniles or at final body size. Measurement of whole body hormone concentrations offers an option for small fishes and whole body cortisol concentrations have now been measured in a range of freshwater and marine species with the technique being suitable both for juveniles of larger species (de Jesus and Hirano, 1992; Barry et al., 1995; Feist and Schreck, 2002; Jentoft et al., 2002), and small adult fish (Pottinger and Calder, 1995; Pottinger et al., 2002; Ramsay et al., 2006). The use of the technique for wild populations requires the capacity to capture fish rapidly without prior disturbance, and some form of endocrine clamping (usually freezing in liquid N₂) to prevent any post-mortem metabolism of corticosteroids. Because of the ubiquity of some species of small fish, there would appear to be considerable potential for their use as environmental sentinels; however, this has yet to be widely applied with respect to corticosteroid status. An exception is the study by Pottinger et al. (2002) demonstrating the potential of three spined stickleback *Gasterosteus aculeatus* as a sentinel species. Captive fish showed predictable stress-induced increases in whole body cortisol, and decreases in RNA:DNA ratios, showing the capacity for environmentally induced changes in stress parameters to be detected in whole body measurements.

There is also unexplored scope for assessment of stress status in rare or difficult-to-capture wild fish by measurement of corticosteroid release to the water. Non-invasive measurement of cortisol release across gills to the holding water has been demonstrated for freshwater fish including rainbow trout (Ellis et al., 2004), carp (Ruane and Komen, 2003), convict cichlids *Archocentrus nigrofasciatus* (Early et al. 2006), and also for Atlantic salmon held in sea water (Ellis et al., 2007). There appears to be no dispute that this technique provides the best option for removing sampling disturbance as an artefact, and it has the added advantage of allowing serial sampling also without disturbance (Scott and Ellis, 2007). However, the usefulness of

the approach in natural situations may be limited by the relative nature of measured steroid concentrations, difficulty in interpreting data from populations of mixed body size and dominance status, and the limited number of natural situations where water volumes will be small, or isolated enough for effective sampling.

Measurement of plasma cortisol from fish captured and sampled rapidly, underwater (Table 3) gives cortisol values that are generally similar to, or lower than those reported from acclimated captive or cultured stocks managed under low disturbance husbandry regimes (eg. Strange et al., 1977; Bry, 1982; Pickering and Pottinger, 1983; Barton et al., 1985; Sumpter et al., 1986; Thorpe et al., 1987; Robertson et al., 1988; Tomasso et al., 1996; Pottinger 1998; Cleary et al., 2000). Baseline values from fish sampled underwater are typically $<10 \text{ ng mL}^{-1}$ and commonly $< 5 \text{ ng mL}^{-1}$ (Table 3), although values of 10s ng mL^{-1} can occur in some species (Pankhurst, 2001). Underwater samples taken from a range of species of wild fish acclimated to a large public aquarium (Table 3) yielded very similar outcomes, suggesting that underwater access to acclimated wild fish may offer an acceptable and more manageable option for sampling some species. Similar baseline levels are recorded from angled fish provided there is a short interval between capture and sampling (Table 3). This suggests that most undisturbed teleost populations are likely to have mean cortisol concentration $< 10 \text{ ng mL}^{-1}$, and that values above this may be indicative of environmental stress, subject to the caveats with respect to sampling discussed above. There are some exceptions to this and these include salmonids where corticosteroid levels change markedly with life history stage (Barton et al., 1985; McBride et al., 1986; Pankhurst and Dedual, 1994; Carey and McCormick, 1998; Pankhurst et al., 2008b) and what appears to be a small number of species that show much higher plasma cortisol levels in unstressed fish. An example is provided by free ranging bluegill sunfish *Lepomis macrochirus* where territorial nesting males typically have plasma cortisol levels in the range $25\text{-}125 \text{ ng mL}^{-1}$ (Magee et al., 2006). A more extreme example is the chub, *Leuciscus cephalus* where resting cortisol levels are typically $50\text{-}100 \text{ ng mL}^{-1}$, and can increase to as high as 1500 ng mL^{-1} during stress. This high cortisol tone in chub appears to be offset by low cortisol receptor affinity (Pottinger et al., 2000). The phenomenon does not appear to be typical of cyprinids generally with roach *Rutilus rutilus* (Pottinger et al., 1999) and carp (Pottinger, 1998) both displaying resting cortisol levels of about 10 ng mL^{-1} .

As discussed in Section 2, there is very little information on natural baselines in chondrosteans and elasmobranchs, arising from the limited number of field studies on chondrosteans, the current limitations on corticosteroid measurements in elasmobranchs and the difficulty in rapidly accessing and sampling large mature fish in both groups. The limited data from wild adult chondrosteans (Semenkova et al., 1999; Bayunova et al., 2002) show cortisol levels at capture that are very much higher than resting levels in cultured or acclimated stocks. This suggests that fish were already stressed at capture, or as in salmonids, corticosteroid status is very strongly determined by life history status. There are no reliable baseline measurements of $1\alpha\text{-OHB}$ from wild sharks, and the use of other stress measures such as acid-base disturbance is often compromised by extended or unknown capture times associated with netting and long-line capture (Manire et al., 2007; Mandelman and Skomal, 2009). Recent studies have examined the effect of timed line capture on plasma lactate level in the small inshore sharks, the white spotted spurdog *Squalus acanthias* and the Australian sharpnose *Rhizoprionodon taylori*. Both species show low ($< 2\text{-}3 \text{ mM}$) plasma lactate values at capture (within 1-2 mins of hooking) and then substantial increases (to $> 20 \text{ mM}$) over extended capture times of up to 2h (Awruch

et al., in review). This suggests that plasma lactate values are a useful first approximation of metabolic stress, provided that the relationship between capture (and length of time of exposure to fishing gear) and sampling is known.

4. Variability in corticosteroid levels

The previous sections generally assessed mean values for defining typical baseline or rested states, and the rates of elevation and recovery following the imposition of a stressor. However, it is clear from studies largely on captive or cultured stocks including rainbow trout and Atlantic salmon (Fevolden et al., 1993; Pottinger and Carrick, 1999a), carp (Tanck et al., 2002), sea bream *Sparus auratus* (Tort et al., 2001), catfish *Clarias gariepinus* (Martins et al., (2006) and striped bass (Wang et al., 2004), that there can be a considerable level of individual variation in both basal and stress-induced plasma corticosteroid levels. Selective breeding experiments have demonstrated that there is a heritable component to at least part of this response (Fevolden et al., 1991, 2003; Pottinger and Carrick, 1999b; Tanck et al., 2002). Subsequent selection for low or high stress responsiveness (corticosteroid response) appears to carry with it a number of associated characteristics including increased locomotor activity and reduced feeding (Øverli et al., 2002), reduced capacity to tolerate multiple stressors (Fevolden et al., 2003), greater likelihood of subordinate behaviour in encounter trials (Pottinger and Carrick, 2001), and reduced food conversion efficiency and growth (Fevolden et al., 2002; Martins et al., 2006) among fish identified as 'high responders'. It is not clear whether these differences arise from co-selection of a series of related traits, or are due to the differential corticosteroid profiles during stress. The adaptive significance of variable responsiveness to stress within a population is suggested to be as a mechanism allowing selection for a range of different stress-coping strategies (Øverli et al., 2002).

Overlaid on any genetic variability in stress response present in a population there is variability related to a number of proximate factors. Diel changes in plasma cortisol levels with peaks early in the scotophase have been recorded in rainbow trout (Bry, 1982; Rance et al., 1982; Laidley and Leatherland, 1988a), brown trout (Pickering and Pottinger, 1983), and carp (Kühn et al., 1986). Juvenile Atlantic salmon showed cortisol peaks in the scotophase for most of the year, but peaks in the photophase during summer (Thorpe et al., 1987). There are also post-prandial rises during the photophase reported for rainbow (Bry, 1982; Laidley and Leatherland, 1988), and brown trout (Pickering and Pottinger, 1983). There is also a seasonal component in some species, which may also be associated with reproductive maturity or life history stage. This is most marked in salmonids with spring smoltification generating large transient increases in basal and stress induced plasma cortisol levels of juveniles (Barton et al., 1985; Thorpe et al., 1987; Carey and McCormick, 1998; Pankhurst et al., 2008b). Adult salmonids also show a sustained increase in cortisol levels in association with sexual maturation and riverine migration (eg. McBride et al., 1986; Pankhurst and Dedual, 1994; Carruth et al., 2000; Westring et al., 2008). There is less information on seasonal change in wild populations of non-salmonid species but there are prespawning increases in plasma cortisol in plaice *Pleuronectes platessa* (Wingfield and Grimm, 1977) and perch *Perca fluviatilis* (Noaksson et al., 2005). In contrast, there is a winter peak in basal cortisol levels in free-ranging snapper in association with gonadal regression (Pankhurst and Sharples, 1992), and no seasonal or maturity-related changes in blue cod *Parapercis colias* captured and sampled

underwater (author's unpublished data) or wild black bream *Acanthopagrus butcheri* (Haddy and Pankhurst, 1999), suggesting that increase in corticosteroid tone with sexual maturation is not a universal feature among teleosts. There is also intriguing recent evidence that an individual's stress history can be a determinant of subsequent corticosteroid status. Brief stress episodes, or cortisol applied to rainbow trout very early in ontogeny (eyed egg, hatching and yolk absorption stages) resulted in a depressed cortisol response to stress at 5 months of age, suggesting that differential stress responses among adults might reflect experience during ontogeny as well as heritable characteristics (Auferin and Gustin, 2008). The mechanisms involved are not currently understood, but similar effects in mammals arise from increased central sensitivity to corticosteroid negative feedback (Meaney et al., 1989; Vallée et al., 1999).

An additional proximate factor that has the capacity to affect corticosteroid status is the behavioural experience of an individual. Paired encounter experiments in captive fish show that dominance is usually rapidly established, and that this is associated with increased plasma cortisol levels in subordinate fish (eg. Ejecke and Schreck, 1980; Laidley and Leatherland, 1988b; Pottinger and Pickering, 1992). However, brown trout allowed to establish dominance hierarchies in an artificial stream showed lower weight in subordinate fish, but no difference in plasma cortisol levels in relation to position in the dominance hierarchy (Sloman et al., 2000). This has led to the cautionary view that outcomes from paired encounter experiments are unlikely to be reflective of more complex behavioural relationships in natural habitats, and that these require more complex test environments for assessment (Sloman and Armstrong, 2002). The limited data available from field studies would appear to confirm this view (see next section).

5. Stress responses in the natural environment

A primary question in the examination of environmental stress is whether animals in the wild typically experience stress over the normal range of activity and environmental conditions. The best field evidence is from studies on birds, and these suggest that probably they don't. Studies on free living populations show that quite harsh environmental conditions and the rigors of reproduction are not necessarily stressful if they are predictable (Wingfield, 1994). Increasing corticosteroid concentrations when conditions do become sufficiently adverse typically modulate behaviour (eg. a shift from nesting to foraging or even refuge seeking) both to ameliorate the stress but also to potentiate recovery and resumption of reproductive behaviour when conditions permit. Events capable of stimulating increases in plasma corticosterone levels include storms and extreme temperatures (Romero et al., 2000). However, even here the relationship may not be straightforward with Arctic passerines being able to cope with storm events during the breeding season but showing stress responses only during the more energetically demanding period of molting (Romero et al. 2000). This has led to the view that events such as migration and reproduction are demanding but not necessarily stressful. Extreme events such as storms encapsulate an 'emergency life history stage' (ELHS) as a response to the unpredictable or extreme event. The ELHS is temporary and maximises survival chances through the associated stress response, only becoming maladaptive if the ELHS persists for too long (Wingfield and Ramenofsky, 1999). The phase of negative energy balance at which an ELHS is triggered is in turn thought likely to be a function of individual body condition.

As in fishes, individual corticosteroid concentrations in free ranging birds can be quite variable. Unstressed Lapland longspurs *Calcarius lapponicus* have resting plasma corticosterone levels of $<5 - \sim 30 \text{ ng mL}^{-1}$ (Romero et al., 2000), values in Adelie penguins *Pygoscelis adeliae* range from $<1 - \sim 11 \text{ ng mL}^{-1}$ at first sampling (Cockrem et al., 2009), and from $<3 - >20 \text{ ng mL}^{-1}$ in Costa Rican rufous-collared sparrows *Zonotrichia capensis costaricensis* (Busch et al., 2010). Cockrem et al. (2009) suggest on the basis of observed correlation between behaviour and stress responsiveness, that low responders (which tend to have proactive ‘personalities’) do best under predictable environmental conditions, whereas high responders (which tend to have reactive personalities) are best adapted for responding to unpredictable events. This is similar to the view expressed by Øverli et al., (2002) that a spread of stress responsiveness in fish populations allows for a range of adaptive or coping strategies in the face of environmental stress. In an examination of studies of 53 species (37 avian, 7 mammalian, 7, reptilian and 2 piscine) where some measure of fitness was correlated with basal corticosteroid concentrations, (Bonier et al., 2009) concluded that there was not a consistent relationship between the two. This was despite a prediction based on the relationship in many species whereby basal corticosteroid levels, and fitness increase and decrease, respectively in the face of environmental challenge, that fitness would decline with increasing basal corticosteroid levels. This further emphasises that the use of corticosteroid levels as predictors of relative fitness requires careful validation in relation to the particular species, population and situation (Bonier et al., 2009).

The level to which natural populations of fish experience stress is difficult to gauge for two reasons. Firstly, there is a very limited number of field studies where free ranging fish have been sampled in ways that allow correlation with pre-capture behaviour and activity, and secondly, the events that might equate to extreme conditions in terrestrial systems (storms and floods) largely preclude sampling, or observation of behaviour at these times. A possible exception to this might be stress associated with reduced flow rates and, or water levels in riverine systems. A series of field studies on the tropical spiny damselfish *Acanthochromis polyacanthus* does offer some insight of the corticosteroid dynamics in free living fish. Plasma cortisol levels in territorial adult fish captured underwater and sampled immediately, ranged between <1 and 42 ng mL^{-1} , and there was no apparent relationship between cortisol level and the time a diver had been in close proximity to the territory (Pankhurst, 2001). Neither was there any relationship between baseline cortisol levels and plasma levels of testosterone (T) and 17β -estradiol in females, or T and 11-ketotestosterone (11KT) in males, despite laboratory experiments showing stress-suppression of sex steroids in both sexes. Earlier studies showed that there was some variation in plasma cortisol levels with behaviour in females but not males, with highest cortisol levels occurring when fish were paired but not yet protecting broods (both sexes of spiny damselfish tend the eggs and then juveniles for several months) (Pankhurst et al., 1999). Treatment of territorial adults with exogenous cortisol had no short-term effect on territorial or guarding behavior, but both control (saline-injected) and cortisol treated fish had become diver negative to the extent that recapture was not possible. Laboratory experiments indicated that the cortisol treatment probably elevated plasma levels to $\sim 100 \text{ ng mL}^{-1}$ (Pankhurst, 2001). Free ranging wild bluegill sunfish show elevated cortisol levels in males engaged in parental care (as noted earlier, up to 125 ng mL^{-1}) but maintain regular cycles of plasma T and 11KT in association with spawning and egg protection (Magee et al., 2006). Treatment of wild largemouth bass *Micropterus salmoides* with cortisol injections that elevated cortisol to levels in

excess of 2500 ng mL⁻¹ had no immediate effect on parental care behaviors by males, but did eventually result in a greater level of nest abandonment (O'Connor et al., 2009). Because of the delay in this effect, it was viewed as most likely resulting from secondary effects of the very high cortisol levels on metabolism and disease resistance, rather than a direct effect of cortisol on reproduction. The conclusion from these studies is that normal reproductive activity is maintained over a range of corticosteroid states, and that moderately elevated plasma cortisol levels in wild fish are not apparently indicative of stress. This is consistent with the emerging view that as in other vertebrates, reproduction in fish is not in itself stressful, and in conditions where stress inhibition of reproduction does occur, other events have usually pushed the animal into an emergency, stress-mediated coping strategy (Schreck, 2010).

The question of whether extreme events modify this pattern of response in a way similar to that described for birds, is harder to assess for the reasons noted earlier, but a field study designed to examine the subsequent effects of extreme events on habitat quality in relation to endocrine status in spiny damselfish had some unexpected results. Spiny damselfish are dependent on structured coral habitat for nesting and effective brood protection (Pankhurst et al., 1999), and it was predicted that fish living in habitats structurally degraded by cyclonic storms and crown-of-thorns starfish *Acanthaster planci* predation on live coral, might be more stressed and have lower reproductive fitness (Pankhurst et al., 2008a). This was in fact not the case, with no measurable effect of habitat quality (coral cover and complexity) on plasma cortisol levels, and highest ovarian fecundity among females from the most degraded sites (where fish population density is low), and poorest reproductive condition among fish at sites of high coral cover and fish density. This was interpreted as an effect generated by competition for limited supplies of current-borne planktonic food among fish constrained by site protection from ranging for food (Pankhurst et al., 2008a). A parallel study in the staghorn damselfish *Amblyglyphidodon curacao* demonstrated a similar effect (Pankhurst et al., 2008c). The message appears to be that the inputs contributing to reproductive fitness are complex, and that the physiological responses to environmental challenge may not always be predictable ones.

6. Stress responses as markers of environmental quality

As noted in previous sections, there is a range of events that can contribute to variability in corticosteroid states that are not necessarily indicative of exposure to stressful conditions. A primary consideration then for the use of corticosteroid status in fishes as an environmental sentinel as suggested by Pottinger et al., (2002), is that the natural range and sources of variability in plasma corticosteroid levels (or any other variable) are known, and that sampling is undertaken in such a way as to not compromise baseline levels through sampling artefacts. An example of the potentially stringent requirements for this is provided by the study by Beckman et al., (2000) characterising the physiological condition of natural populations of juvenile chinook salmon in the Yakima River drainage. Responses displayed a seasonal component, changes in relation to stage of smoltification, and possible environmental and genetic differences related to where in the river system the fish came from. The capacity to detect changes arising from environmental degradation, and particularly at an early enough stage to allow potential for intervention, is likely to need a clear understanding of all of the above processes and factors.

A second requirement is that environmental changes activate the HPI axis. A reasonable prediction based on laboratory assessment of the effects of water quality

parameters on stress is that adverse changes in factors such as temperature, dissolved O₂ and CO₂, pH, alkalinity and water hardness are likely to be reflected in alterations in survival (Portz et al., 2006). However, systematic investigation of how parameters affect the HPI axis or other metabolic variables is not available to the extent that the stress response can reliably be used as a diagnostic tool for water quality. There are also some caveats to the potential usefulness of corticosteroid status as an indicator of environmental stress. Some chemical factors contributing to poor water quality can themselves suppress corticosteroid responses (Pickering and Pottinger, 1987), and the effects of temperature change may be reflected in elevated cortisol levels (eg. Nakano et al., 2004) but may also have significant effects that are not apparent through HPI axis activation. In a series of experiments examining the inhibitory effects of elevated temperature on reproduction in rainbow trout and Atlantic salmon (reviewed in Pankhurst and King, 2010), we have not detected any increases in cortisol levels in thermally challenged fish which show substantial reproductive compromise (N.W., Pankhurst, H.R. King and A. Elizur, unpublished data). Assessment of stress responses as sentinels for the presence of aquatic pollutants may also have limited utility as a result of the effect of exposure to a number of toxicants being to attenuate the responsiveness of the HPI axis (Hontela et al., 1992; Hontela, 1998; Norris et al., 1999). The effect is thought to arise from desensitisation of the HPI axis as the result of chronic exposure.

A final requirement for the use of stress as an indicator of environmental change or quality is the need for temporally appropriate sampling. As noted in the discussion of effects in birds, the significant effects of environmental stress are usually exercised on behaviour, and are usually transient. The behaviour-modulating effect of CRF demonstrated in fish (Clements et al., 2002; Clements and Schreck, 2004; Lowry and Moore, 2006) mean that stress-induced behaviour change could happen quite early in a stress event. For example, we don't know what happens to brooding spiny damselfishes in extreme marine weather conditions but it is clear that brood abandonment by just one parent results in rapid predation of the brood (Nakazono, 1993; Pankhurst et al., 1999). Refuge seeking by brooding parents is likely to result in brood loss but this would not be apparent from adult corticosteroid levels after the storm event. Detection of such events that are corticosteroid mediated will require sampling either with the onset of, or during extreme events. In this context, direct observation of stress-related modifications of behaviour such as cessation of feeding and reproductive behaviour, increased activity or shelter seeking (Scheck et al., 1997) may be the most useful proximate measure of response to environmental stress, where conditions permit observation.

7. Concluding remarks

The increasing availability of cultured fish as a result of aquaculture and fishery management initiatives has provided an excellent platform for the development of understanding of the physiology of stress in fish. However, with this comes the risk of inappropriate extrapolation of this understanding to wild populations, about which we still know comparatively little. What is increasingly understood is that the generally simple 'linear' stress paradigms explored in laboratory contexts are not always indicative of the much more complex social, physiological and physical interactions that occur in the natural environment. Data from avian studies and a limited number of examinations of fish show that the stress response has major role in responding to unpredictable or extreme environmental events, and also has the potential to signal

changes in environmental quality. For this potential to be realised, there is an urgent need to substantially improve our understanding of stress and its effects in the natural environment.

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Table 1.

Time for significant increases in plasma cortisol following exposure to stressors, ranked by temperature.

Species	Time (mins)	Temperature (°C)	Source
<i>Pagothenia borchgrevinki</i>	60	-1.9	Ryan (1995)
<i>Onchorhynchus mykiss</i>	10	5.0	Sumpter et al. (1985)
<i>Oncorhynchus kisutch</i>	8	9.0	Sumpter et al. (1986)
<i>Oncorhynchus tshawytscha</i>	10	8.0	Strange et al. (1977)
<i>Oncorhynchus mykiss</i>	5	10.0	"
"	10	13.0	Sumpter et al. (1985)
<i>Hemitripterus americanus</i>	120	10.0	Vijayan and Moon (1994)
<i>Rhombosolea tapirina</i>	13	12.0	Barnett and Pankhurst (1998)
<i>Acanthopagrus butcheri</i>	>5<15	15.0	Haddy and Pankhurst (1999)
<i>Sciaenops ocellatus</i>	15	19.5	Robertson et al. (1988)
<i>Macquaria ambigua</i>	30	20.0	Carragher and Rees (1994)
<i>Pagrus auratus</i>	30	19.0-20.0	Pankhurst and Sharples (1992)
<i>Scorpius violaceus</i>	10	19.0-21.0	Pankhurst et al. (1992)
<i>Parapercis colias</i>	20	21.0	Author's unpublished data
<i>Stizostedion vitreum</i>	<15	21.0	Barton and Zitzow (1995)
<i>Sciaenops ocellatus</i>	<15	24.0-27.5	Thomas and Robertson (1991)
<i>Acanthochromis polyacanthus</i>	>5<10	28.0	Begg and Pankhurst (2004)
<i>Hemigymnus melapterus</i>	5	28.0-30.0	Grutter and Pankhurst (2000)
<i>Morone saxatilis</i>	2.5	26.0-32.0	Tomasso et al. (1996)
<i>Oreochromis mossambicus</i>	3	n.s. ¹	Balm et al. (1994)

¹n.s. not specified.

Table 2.

Plasma 1 α -hydroxycorticosterone (1 α -OHB) levels reported for elasmobranchs.

Species	Plasma 1 α -OHB (ng mL ⁻¹)	Holding conditions ¹	Source
<i>Raja clavata</i>	15.0	n.s.	Idler and Truscott (1969)
	<0.8 – 139.0	WC	Kime (1977)
<i>R. radiata</i>	2.0 – 20.0	WC	Idler and Truscott (1968)
	16.0-43.0	WC	Idler and Truscott (1969)
<i>R. erinacea</i>	9.0	n.s.	"
<i>R. laevis</i>	9.0	n.s.	"
<i>R. ocellata</i>	4.0	n.s.	"
<i>Scyliorhinus caniculus</i>	1.0 – 7.8	WC	Kime (1977)
<i>S. canicula</i>	~ 4.0	WC	Hazan and Henderson (1985)

¹WC = wild fish held under captive conditions for varying periods; n.s. = not specified.

Table 3.

Plasma cortisol levels at capture in wild fish sampled underwater, or following rapid line capture.

Sampling method	Species	Sampling time (min) ¹	Plasma cortisol (ng mL ⁻¹)	Source
Underwater capture and sampling	<i>Pagrus auratus</i>	<1	1.7-8.0	Pankhurst and Sharples (1992)
	<i>Parapercis colias</i>	<1	0.9-2.1	Author's unpublished data
	<i>Chromis dispilus</i>	<1	3.0	"
	<i>Coris sandageri</i>	<1	1.3	"
	<i>Bodianus vulpinus</i>	<1	1.2	"
	<i>Rhombosolea tapirina</i>	<1	3.9	Barnett and Pankhurst (1998)
	<i>Hemigymnus melapterus</i>	<1	3.0	Grutter and Pankhurst (2000)
	<i>Acanthochromis polyacanthus</i>	<1	11.0	Pankhurst (2001)
	<i>Lepomis macrochirus</i>	<5	25.0-125.0	Magee et al. (2006)
Underwater sampling (aquarium) ²	<i>Coris sandageri</i>	<1	1.2	Author's unpublished data
	<i>Bodianus vulpinus</i>	<1	2.1	"
	<i>Scorpius violaceus</i>	<1	2.2	"
	<i>Chromis dispilus</i>	<1	1.2	"
Line capture	<i>Scorpius violaceus</i>	1	3.3	Pankhurst et al. (1992)
	<i>Acanthopagrus butcheri</i>	<5	1.9-2.8	Haddy and Pankhurst (1998)
	<i>Oncorhynchus mykiss</i>	<5	19.0-34.0	Pankhurst and Dedual (1994)
	<i>Cynoscion nebulosus</i>	<5	6.2	Safford and Thomas (1987)
	<i>Plectropomus leopardus</i>	<1	~4	Frisch and Anderson (2005)
	<i>Plectropomus maculatus</i>	<1	~3	"
	<i>Macquaria ambigua</i>	<1	0.9	Carragher and Rees (1994)

¹Time from first encounter with capture equipment, to sampling. ²Wild fish sampled by divers from a large (200m³) public aquarium