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The effects of stocking density on fish welfare

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Abstract

The welfare of intensively farmed fish is a subject of increasing interest and one of the principal areas of concern is stocking density. Several studies have examined the effects of density on the welfare of farmed fish, and have found it to be a source of chronic stress with commonly reported effects including reduced growth rates, alterations in the physical condition and health of fish, and the activation of stress responses. Such changes in the biological and physiological systems of fish are indicative of a reduced welfare status. However due to pronounced interspecies variations in behavioural and physiological requirements, the way in which stocking density affects various aspects of welfare in farmed fish is strongly species-specific, and in some cases life stage dependent. The combination of a range of indicators to assess the effect of density on fish welfare is the most reliable method to determine whether stocking density has a detrimental impact on the welfare of intensively farmed fish.

Key words: stocking density; welfare; stress indicators; growth; fin condition; immune status.

Introduction

It is estimated that global production from capture fisheries and aquaculture supplied the world with around 110 million tonnes of fish in 2006. Of this total, aquaculture accounted for 47 % (Food and Agriculture Organisation of the United Nations, 2008). Aquaculture continues to be the fastest growing animal food producing sector, and has overtaken capture fisheries as a fish food source (Food and Agriculture Organisation of the United Nations, 2008). Partly due to this rapid expansion, the welfare of intensively farmed fish has received increased public, commercial and governmental attention and has become a much debated topic (Farm Animal Welfare Council, 1996; FSBI, 2002; Lymbery, 2002; Ashley, 2007). Fish welfare is an important issue for the industry, in terms of public perception, product acceptance, marketing and also in relation to quality, quantity and product efficiency (Broom, 1998; FSBI, 2002). However many animal rights pressure groups have stated that there are conflicts between welfare and current farming practices, whereby existing procedures are seemingly associated with diminished welfare (FSBI, 2002; Lymbery, 2002).

Although there is no universally accepted definition of welfare, it is commonly seen to represent the physical and mental state of an animal in relation to its environment (Farm Animal Welfare Council, 1996; Lymbery, 2002) thereby reflecting its well-being, health, quality of life and an absence of suffering (North *et al.*, 2006). The concept of suffering is central to the idea of animal welfare and there has been ongoing scientific debate concerning the capacity of fish to suffer (Rose, 2002; Chandroo *et al.*, 2004; Braithwaite and Boulcott, 2007), as traditionally the concept has only been applied to species that have a higher level of cognition when compared to fish (Ashley, 2007). Some scientists have stated that fish lack the essential brain region or functional equivalent, to be able to experience pain and fear (Rose, 2002). Others have suggested that there is physiological, behavioural and anatomical evidence that make it conceivable that nociception in fish is experienced, and that there is potential for fish to experience suffering (Sneddon, 2003; Sneddon *et al.*, 2003; Chandroo *et al.*, 2004; Ashley, 2007; Braithwaite and Boulcott, 2007).

Throughout the aquaculture industry fish are subjected to many different sources of stress due to husbandry practices, such as handling, capture and confinement; by far the most common source is inappropriate stocking density (Rotllant *et al.*, 1997). Just as there are concerns over the intensity of terrestrial livestock production (Dawkins, 2006), the stocking densities used in commercial fish culture have been highlighted as an area of particular concern by the government's advisory body the Farm Animal Welfare Council (1996), to be a "crucial factor affecting fish welfare".

When farming terrestrial animals, minimum spatial areas are stipulated to provide for the animal's needs (Anon, 1995), however with regards to fish culture there are currently no regulations stating the densities at which fish should be farmed (Lymbery, 2002). The term stocking density relates to the concentration at which fish are initially stocked (Ruane *et al.*, 2002), and relates to the density of fish at any point in time (Ellis *et al.*, 2002). It is defined in terms of kilograms of fish per cubic meter of water, reflecting the three dimensional environment inhabited by many species of fish including trout and salmon. For bottom dwelling flatfish, stocking is more often given as mass of fish per square meter (Lymbery, 2002).

Stocking density has become widely recognised as an important husbandry factor in intensive fish culture due to it representing a potential source of chronic stress, which may have adverse effects on the physiological, health and/or behavioural status of the individual fish involved, for example reductions in reproductive output and changes in disease resistance (Ashley, 2007). These indicators can in turn be used as signs of compromised welfare (Wedemeyer, 1997; Ellis *et al.*, 2002; Huntingford *et al.*, 2006). This review will explore how stocking densities used in intensive fish farming may be a potential source of stress and the consequences of this for fish welfare, in particular its effect on the stress response found in fish, changes in fish health and condition, and alterations in growth rates.

Evidence for effects of stocking density on fish welfare

Stress hormones

Much of our understanding of how fish respond to adverse environments comes from the extensive literature on the biology of stress (Huntingford *et al.*, 2006). In common with other vertebrates, fish exhibit a range of physiological and behavioural strategies aiding them to deal with a destabilising stimulus or stressor (FSBI, 2002). This response is known as the generalized stress response (Iwama, 2007), and is often used as an indicator of impaired welfare. Consequently measures of physiological stress responses feature predominantly in studies regarding welfare (Huntingford *et al.*, 2006; Ashley, 2007). This stress response is considered to be part of an adaptive strategy to cope with a perceived threat to homeostasis (Sutanto and de Kloet, 1994), and has been categorized into primary, secondary and tertiary responses (FSBI, 2002). The initial or primary response comprises a neuroendocrine response, which involves the release of catecholamines and the activation of the hypothalamic-pituitary- interrenal (HPI) axis. Corticotropin releasing factor from the hypothalamus acts on the pituitary to synthesise and release adrenocorticotropic hormone, which subsequently stimulates the synthesis and mobilisation of glucocorticoid hormones (cortisol in teleosts) from the interrenal tissue located in the head kidney (Wendelaar Bonga, 1997; FSBI, 2002; Huntingford *et al.*, 2006; Ashley, 2007; Iwama, 2007).

The size and duration of stress-induced elevations in plasma cortisol levels are usually proportional to the duration and severity of the stressor involved (FSBI, 2002), with recovery from a short term, acute stress taking a matter of hours (Pickering and Pottinger, 1989). Some studies have shown that elevated cortisol levels generally persist during continuous chronic stress, of which stocking density can be a source (Pottinger and Moran, 1993; FSBI, 2002). Previous studies on red porgy (*Pagarus pagarus*) reared at 20 kg m^{-3} were found to have higher cortisol levels than fish kept at 7 kg m^{-3} (Rotllant *et al.*, 1997). Findings from another sparid, the gilthead sea bream (*Sparus aurata*) showed similar responses with individuals reared at 40 kg m^{-3} showing higher cortisol levels compared to fish held at 10 kg m^{-3} (Montero *et al.*, 1999). Furthermore the duration of the stocking stress involved and its effect on plasma cortisol levels has been shown for a number of salmonid species. Mazur and Iwama (1993) found elevated plasma cortisol concentrations in chinook salmon (*Oncorhynchus tshawytscha*) after 33 days of crowding. Similarly plasma cortisol concentrations were found to remain elevated for up to 4 weeks in salmonids held at high densities (Pickering and Pottinger, 1989; Schreck, 2000). As well as the evidence supporting the idea that high stocking densities cause stress and in turn elevated cortisol levels, alternative studies have found no significant differences in cortisol levels in response to density. Sammouth *et al.* (2009) reported no significant difference in cortisol levels in fish kept at 10, 40 or 100 kg m^{-3}. Similar results were reported for European sea bass (*Dicentrarchus labrax*) reared at 21 kg

m^{-3} (Di Marco *et al.*, 2008) and 45 kg m^{-3} (Marino *et al.*, 2001). The discrepancies mentioned above could be related to pronounced interspecies variations in the physiological requirements of each species. They could also be due to experimental factors other than density, such as water quality variation (Ellis *et al.*, 2002).

The secondary stress response is made up of the physiological and biochemical effects associated with stress, and is mediated by stress hormones (Iwama, 2007). These stress hormones cause the activation of metabolic pathways which result in changes in haematology and blood chemistry (Iwama, 2007). Primary and secondary stress responses are generally short term consequences of acute challenges (FSBI, 2002), however when a stress is prolonged and the individual has no means of escape, tertiary effects become apparent (Schreck, 2000). Welfare measures in aquaculture are largely associated with the tertiary effects of stress response, and include alterations in growth rates, immune function and reduced physical condition, all of which are discussed below (Barton *et al.*, 2005; Barton and Iwama, 1991; FSBI, 2002; Kristiansen *et al.*, 2004; Pickering and Pottinger, 1989).

Health and condition

The FSBI (2002) function based definition of animal welfare, centres on the fish's ability to adapt to its environment. Here good welfare requires the animal to be in good health and physical condition, with its biological systems functioning appropriately. Stocking density has been shown to affect the health and condition of fish, therefore these indicators can be used as fundamental measures of the welfare of farmed fish (Bjornsson, 1994; Wedemeyer, 1997; Ellis *et al.*, 2002; Barton *et al.*, 2005; Falahatkar *et al.*, 2009).

Immune function

Stocking density causes changes in the immune system and its impairment can lead to disease or death (Maule *et al.*, 1989; Mazur and Iwama, 1993; Rotllant *et al.*, 1997). Fish subjected to periods of chronic stress show a decreased ability to fight against pathogens, and immunosupression or immunodepression has been described in such circumstances (Maule *et al.*, 1989; Pickering and Pottinger, 1989; Di Marco *et al.*, 2008). In order to monitor the immune status in fish under stressed conditions, the non- specific or natural immune response is generally monitored (Rotllant *et al.*, 1997) as this system does not depend on prior disease challenges and is effective against a range of antigens (FSBI, 2002). A wide number of immune responses can be monitored after periods of stress, for example the haemolytic and agglutinating activity of fish serum has been used for such purposes (Tort *et al.*, 1996; Rotllant *et al.*, 1997). The haemolytic activity of serum is based on the Alternative Complement Pathway (ACP). It has been shown that ACP is an important non-specific defence mechanism, and is involved in the clearance of bacteria, viruses and fungi (Sunyer and Tort, 1995). Natural haemagglutinins have also been reported to play a role in the non-specific defence in fish, as they display a high agglutinating activity against rabbit erythrocytes (RaRBC) and different bacteria (Sunyer and Tort, 1995). Lysozyme concentration is also used to test the response of an organism to infection. Its inter organ distribution in fish (found mainly in mucus, blood and lymphomyeloid tissues) suggests its use as an antimicrobial defence mechanism (Rotllant *et al.*, 1997). Finally the number of circulating lymphocytes and the concentration of total immunoglobulin are alternative parameters used as indicators of immune changes after stress (Maule *et al.*, 1989; Tort *et al.*, 1996).

Several studies have used a combination of the immune indicators mentioned above to explore whether crowding stress affects the non-specific immune response in fish. Rotllant *et al*. (1997) measured the haemolytic and lysozyme activity of serum, total immunoglobulin concentration and number of circulating lymphocytes in red porgy in response to crowding stress after a 23 day period. Results indicated that immunodepression was found to be evident following exposure to this chronic stress which was shown in the decrease of ACP levels and circulating lymphocytes. Both corticosteroids and catecholamines influence immune status (Rotllant *et al.*, 1997), and catecholamines in mammals suppress lymphocyte proliferation to mitogens (Khansari *et al.*, 1990), and also induce their redistribution (Landmann *et al.*, 1984). In fish however, effects on immune function have been linked with corticosteroids (Maule and Schreck, 1991; Mazur and Iwama, 1993). Furthermore corticosteroid receptors have been found in fish lymphocytes, and changes in glucocorticoid receptors in both leukocytes and lymphoid tissues after chronic stress have been shown (Maule and Schreck, 1991). An alteration in leukocyte numbers can indicate poor welfare as a reduction in their abundance due to increasing density can theoretically increase a fish's susceptibility to disease, reduce its clotting rate and hence its defence against physical injury (Pickering and Pottinger, 1989). The concentration of immunoglobulin is affected by stress and corticosteroids are known to have a suppressive effect on the humoral antibody production in fish (Maule *et al.*, 1989; Mazur and Iwama, 1993; Rotllant *et al.*, 1997).

One of the consequences of stress induced changes in immune function is that chronic exposure to adverse situations makes fish more vulnerable to disease and death (Pottinger and Moran, 1993; Wedemeyer, 1997; FSBI, 2002; Ashley, 2007). Pickering and Pottinger (1989) administered cortisol to salmonid fish and reported mortalities due to increases in bacterial and fungal pathogens. Other studies have reported incidences of stress mediated bacterial diseases, such as furunculosis and vibriosis (Plumb, 1994). Therefore the negative effects that density has on immune status in fish, lend themselves to being a sensible indicator for measuring reduced welfare.

Fin condition

With higher stocking densities comes the issue of increased crowding stress, and injuries such as fin damage gained by these adverse conditions have been used as indicators of poor welfare (FSBI, 2002). Fin condition has been assessed by comparing the lengths of fins in relation to body length (Kindschi, 1987; Miller *et al.*, 1995) or by subjective classification of the extent of damage (Boydstun and Hopelain, 1977; Mäkinen and Ruohonen, 1990). Assessments have been made on anal, pectoral, pelvic, dorsal, caudal and adipose fins (Boydstun and Hopelain, 1977; Kindschi, 1987). The exact cause(s) of increased fin damage at higher stocking densities is unknown (North *et al.*, 2006), but suggestions include: aggressive and/or accidental nipping during feeding and abrasion against conspecifics, or the walls of the rearing units the fish are held in (Mäkinen and Ruohonen, 1990; Winfree *et al.*, 1998). Intensive aquaculture studies on the frequency and severity of fin damage and its relation to stocking density have focused on salmonids (Ellis *et al.*, 2002; Turnbull *et al.*, 2005; North *et al.*, 2006; Person-Le Ruyet, 2009). In species where aggression is common, crowding stress can increase competition between individuals and in turn increase instances of fin damage. North *et al.* (2006) reported increased levels of fin damage at densities of 40 and 80 kg m^{-3} compared to 10 kg m^{-3} treatments, and suggested that the potential for aggressive or accidental damage increased due to higher numbers of fish. This trend has also been shown for Arctic charr (*Salvelinus alpinus*) (Damsgard *et al.*, 1997), Atlantic salmon (*Salmo salar*) (Turnbull *et al.*, 2005), and rainbow trout (*Oncorhynchus mykiss*) (Ellis *et al.*, 2002). Conversely Fairchild and Howell (2001) conducted a study on a flatfish species, winter flounder (*Pseudopleuronectes americanus*), and found no difference in caudal fin damage between different stocking densities and suggested that aggressive behaviour was not adversely affected by density. Although this study conflicts with the idea that density negatively affects fin condition in salmonids, it should be viewed with caution as there have been only a small number of studies carried out on the affects of density on fin damage in other flatfish species.

Growth rate

Many of the effects of stress described previously cause reduced energy intake and increased energy utilisation, so prolonged activation of the HPI axis is likely to indirectly reduce growth through a negative effect on energy balance (Ellis *et al.*, 2002; Huntingford *et al.*, 2006). In addition the secretion of growth hormones in fish is suppressed during periods of stress (Pickering *et al.*, 1991; Farbridge and Leatherland, 1992), therefore stress also has a direct influence on the mechanisms controlling growth (FSBI, 2002). Since these alterations can be interpreted as adverse changes to normal function, change in growth rates have been used as an indicator of reduced welfare (Ellis *et al.*, 2002).

Various methods have been used to quantify the effect of stocking density on growth, described as mean weight or length at the end of an experimental period, mean weight/length gain, individual or mean specific growth rates (Ellis *et al.*, 2002). All aforementioned methods are equally valid ways of measuring the change in size of individuals over time at different densities (Ellis *et al.*, 2002; Correa and Cerqueira, 2007; d'Orbcastel *et al.*, 2009). Growth rates in fish are flexible and naturally variable over short periods of time, but provided that estimates of expected growth rates are available, then prolonged low growth may be used as an indicator of chronic stress (Huntingford *et al.*, 2006).

Studies in the laboratory and aquaculture settings show that the effect of stocking density on growth performance varies between species. High stocking densities have resulted in reduced growth rates in a number of fish species such as European sea bass (Saillant *et al.*, 2003), rainbow trout (Holm *et al.*, 1990; Ellis *et al.*, 2002), and Atlantic cod (*Gadus morhua*) (Lambert and Dutil, 2001). However this is not an explicit trend and studies involving fat snook (*Centropomus parallelus*) (Correa and Cerqueira, 2007), Atlantic salmon (Kjartansson *et al.*, 1988), and Arctic charr (Brown *et al.*, 1992) have shown increasing or unaffected growth rates.

In a number of flatfish species the tolerance to high densities has been shown to be stage dependent, in a study involving Atlantic halibut (*Hippoglossus hippoglossus*), Greaves and Tuene (2001) found that small juvenile individuals farmed at higher densities had better growth rates in comparison to larger more mature individuals. Howell (1998) found increasing density had no negative effect on the growth rates of turbot (*Scophthalamus maximus*), however Irwin *et al.* (1999) found reduced growth rates of smaller turbot with increasing density. Tolerance to increasing stocking density and its association with life stage dependence has been found for the African catfish (*Clarias gariepinus*). Published data on the growth performance of African catfish when exposed to different stocking densities is similar to the flatfish examples with contradictory outcomes being reported. Several studies on African catfish larvae have shown negative effects of increasing stocking density, reflected by decreased growth performance (Hecht and Appelbaum, 1988; Haylor, 1991; Hossain *et al.*, 1998). Alternative studies on juveniles have found a positive effect of increasing density, reflected by increased growth (Hecht and Appelbaum, 1988; Kaiser *et al.*, 1995; Hecht and Uys, 1997), or no effect of density (Hengsawat *et al.*, 1997; van de Nieuwegiessen *et al.*, 2008).

Conditions under which all the aforementioned studies involving growth rates were carried out were highly variable, and it has been suggested that stocking density is not the only possible cause for differences in growth rates; environmental stressors such as altered pH, reduced dissolved oxygen and salinity have been used to explain changes in growth rate (Huntingford *et al.*, 2006). Another issue to be considered is that many of the studies mentioned used low stocking densities (Hecht and Appelbaum, 1988; Haylor, 1991; Kaiser *et al.*, 1995) that were not comparable to commercial aquaculture situations, therefore, making direct comparisons between experimental and commercial situations problematic.

Conclusion

This review has examined the effects density has on the stress response, growth rate, health and condition of intensively farmed fish. It has shown that effects of density are not easily predicted due to pronounced interspecies variation in physiological and behavioural requirements, and shows that the way in which density affects various aspects of welfare of farmed fish is strongly species-specific (Damsgard *et al.*, 1997; Fairchild and Howell, 2001; Ellis *et al.*, 2002; FSBI, 2002).With different welfare indicators individually showing both negative and positive effects of stocking density, the approach of using a range of indicators are combined using multivariate analysis to produce a single welfare score (e.g. Turnbull *et al*., 2005; Saxby *et al*., 2010) seems a sensible step forward in investigating whether density affects fish welfare. Using this method for future studies on welfare will hopefully provide stronger conclusions as to whether stocking density adversely affects the welfare of intensively farmed fish.

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