Pain and stress responses in farmed fish

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Summary

Farming fish for human consumption continues to expand as an industry and, with this increasing interaction with captive fish populations, there is now a growing interest in determining how to create good welfare for the fish we farm. This article summarises recent advances in our understanding of pain and stress responses in fish and how these relate to farmed fish welfare. Over the last decade several studies have examined whether or not fish feel pain, how aversive the experience is, and how such experiences may be mitigated through the use of analgesics. The basic neural mechanisms that enable the detection of tissue damage, i.e. nociceptive mechanisms, appear to be broadly conserved from fish through to birds and mammals, however, there is debate about the extent of the negative feelings associated with pain and whether these are truly experienced by fish. The stress response that helps fish to cope with various challenges also appears to be largely conserved across vertebrates, and the physiological changes that occur in response to acute and chronic stress in fish are similar to those described for mammals. Therefore, fish appear to have the innate ability to experience negative states such as pain and stress in a way analogous to that experienced by other vertebrates. There are multiple situations in which farmed fish may experience pain and stress and there is now a growing recognition that, to deliver appropriate welfare, on-farm practices and procedures will have to change. It is also the case that the welfare requirements of the different fish species that we farm vary, with some species coping better in captive rearing environments than others. The topic of fish welfare is relatively new and more research on stress responses, allostasis, pain thresholds and analgesics is required to help promote good fish welfare.

Keywords

Allostasis – Farmed fish – Pain – Stress – Suffering – Welfare.

Introduction

To provide good welfare for the fish we farm we need to understand how they respond to stressful or noxious situations. Breeding and rearing fish in hatcheries and then transferring them to ponds, raceways, or large cages puts fish into situations that they would never normally experience if they were living in the wild. How the fish adjust to situations such as being handled as they are moved from one enclosure to another, living at high densities, being subject to constant light regimes to stimulate growth or being treated with vaccines and other pharmaceuticals depends on their capacity to adapt to the captive environment. The growing recognition that fish do experience pain and have an affective state that is altered under stressful conditions has led to research focused on the welfare of farmed fish. This review addresses some of the recent advances that we have made in understanding fish pain and stress processes.

The capacity of fish to experience pain

In vertebrates, when an injury to the body occurs, specialised receptors, known as nociceptors, detect the damage. A signal about the injury is then relayed through specialised nerve fibres (A-delta and C fibres) to the spinal cord where reflexive responses may be triggered, some of which involve physiological changes, including the activation of various stress responses that are described in more detail below. From the spinal cord, information may then be transmitted to the brain where specific areas process it, leading to various behavioural and physiological responses.

There are now several systematic studies that have investigated the capacity for ray-finned, bony fish to experience pain (1, 2, 3). Much of the recent research has focused on species of commercial interest, such as rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*), as well as other widely available species such as goldfish (*Carassius auratus*) (1, 3, 4, 5, 6).

Evidence of nociception

Investigations with rainbow trout have found several kinds of nociceptor to be present in the skin and in the cornea of the eye, and have discovered that different forms of receptor respond to different sorts of tissue damage (mechanical injury such as crushing; chemical stimulation, such as exposure to acidic solutions; and thermal damage from increased temperatures) (1, 6, 7, 8, 9). A-delta and C fibres have been isolated and described in nerves such as the trigeminal nerve that innervates the head of the fish, and in nerves innervating the tail fin (1, 6, 10). Thus, ray-finned, bony fish have a nociceptive system that is very similar to the nociceptive systems of mammals and birds.

However, some differences do exist, for example, terrestrial vertebrates possess cold-sensitive nociceptors, but these have not yet been found in fish (6, 11) and fewer C fibres have been found in fish than in mammals and birds (1, 10, 11). The significance of this difference in C fibre number is unclear. However, it is speculated that it is associated with shorter-lived pain responses in fish, because in birds and mammals C fibres act over a longer time course compared to the responses detected through A-delta fibre stimulation, but this has not been formally investigated.

Evidence of higher-order cognitive processing of nociceptive inputs

After tissue damage has been detected and the specialised fibres have passed a signal to the brain, higher-order cognitive processes occur. These induce changes in the motivational and affective states of the fish, for example, decreased appetite, reduced swimming activity and changes in fear responses (1, 2). Various behavioural changes have also been reported, for example, fish pay attention to the affected part of their body and rub it against solid structures in the local environment (1, 12). Recording electrodes in different parts of the brain of rainbow trout and goldfish have shown that poking a pin into the skin just behind their operculum generates a nociceptive signal that triggers activity in the cerebellum, tectum and telencephalon (forebrain) of both species (3), indicating that nociceptive impulses are subject to processing in a number of different brain regions.

While fish do not possess a layered neocortex, there are specialised subdivisions within the telencephalon, such as the dorsal lateral region, which performs a role similar to the hippocampus found in mammalian and avian brains (13, 14, 15, 16). In fish, the dorsal lateral region is involved in learning and memory, and is essential for fish to learn spatial and temporal associations (16, 17). However, whether nociceptive information is processed within this specific area of the telencephalon is not yet known.

In order to have some understanding of the aversive feelings that accompany pain and, consequently, to experience its psychological and emotional aspects, animals must have a capacity for mental awareness. Although several reviews have argued that a number of fish species have such mental capacities (18, 19, 20, 21, 22, 23), others suggest that this capacity is not possible in fish owing to the fact that their brains are less complex than those of mammals (24, 25). Yet, as we continue to discover more about fish neuroanatomy and neurophysiology, it is becoming increasingly accepted that many species of fish do have brains that are sufficiently complex to support mental representations and an understanding of temporal relationships, and that together these help to generate flexible behaviour and complex decision-making. Likewise, it is becoming increasingly accepted that fish do have experiences that relate to negative affective states, such as suffering (26).

For example, Sneddon *et al*. (2003) empirically examined the capacity for pain awareness in fish by using a paradigm previously devised to test for changes in awareness in birds that had ankle joint inflammation (2, 27). Earlier experiments with both mammals and birds reported a change in sensitivity to pain when animals were put into a novel or a fear-inducing environment (28, 29, 30). The change to the environment can alter the attention the animal gives to the pain in two main ways: it may show a decreased sensitivity to pain (hypoalgesia) as it focuses on its new situation; or, if the experience of the pain commands a significant part of the animal's attention, its response to the changed environment may be impaired.

Sneddon *et al*. (2003) used this premise to investigate how trout given a noxious (acetic acid solution) injection subcutaneously into the snout responded to a brightly coloured novel object placed into their home tank (2). Trout usually express strong avoidance of novelty and under normal circumstances keep away from novel objects, and indeed the control fish that were injected with a saline solution did avoid the novel object. However, the trout treated with acetic acid had an impaired neophobic response and failed to express the typical avoidance behaviour. This effect was absent and normal avoidance responses were observed when the fish were given an analgesic at the same time as they received the acetic acid injection (2). Together, these observations indicate that at least some fish species we keep captive on farms are likely to experience negative feelings associated with pain, and therefore that farmed fish welfare is a real phenomenon that we should address (23, 31).

Fish stress response

General overview of the stress axis and its biological relevance

Vertebrates possess a suite of adaptive behavioural and physiological strategies that have evolved to cope with destabilising challenges, or stressors (32). In common with other vertebrates, therefore, fish respond to environmental challenges with a series of adaptive neuroendocrine adjustments that are collectively termed 'the stress response'. These induce reversible metabolic and behavioural changes that enhance the ability of fish to overcome or avoid challenges, and, in the short-term at least, this is beneficial. In contrast, prolonged activation of the stress response is damaging and leads to immunosuppression, reduced growth and reproductive dysfunction (see below).

The neuroendocrine stress response in fish is virtually identical to that of mammals and is mediated by the hypothalamic-pituitary-interrenal (HPI) axis. Perception of a stressor by a fish initiates a rapid, neurally stimulated release of catecholamines from the chromaffin tissue in the head kidney. This is accompanied by release of corticotropin-releasing hormone (CRH) from the hypothalamus. The CRH system plays a key role in the coordination of the physiological response to stress in vertebrates (33). Corticotropin-releasing hormone promotes the release of adrenocorticotrophic hormone (ACTH) by the pituitary gland and subsequent synthesis and secretion of cortisol by the interrenal tissue. Cortisol then binds to mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) in target tissues, including the brain, promoting further behavioural and physiological responses. The hormone cortisol plays an important role in allostasis, i.e. the maintenance of stability. Under baseline conditions, cortisol allows various hormones to function appropriately, but it also has a regulatory role allowing animals to recover from stressors (34). The MR and GR function to regulate cortisol levels. Mineralocorticoid receptors are sensitive to low levels of cortisol and they influence fluid balance within the body, cortisol negative feedback over daily cycles and glucose metabolism (35). Glucocorticoid receptors have a lower affinity for cortisol, thus detect it when it is at high concentrations, and so are intricately involved in the stress response and subsequent recovery (34). Cortisol concentrations return to pre-stress levels within hours of exposure to a brief stressor, but they can remain elevated during continuous, chronic stress (however, see below).

Where fish cannot escape a stressor, or where episodic or intermittent stressful stimuli persist for a period, prolonged activation of the stress response has deleterious consequences. Indicators associated with the response to chronic stress, including physiological outcomes, disease status and behaviour, provide a potential source of information on the welfare status of a fish. These include loss of appetite, impaired growth and muscle wasting, immunosuppression, suppressed reproduction and reduced cognitive ability. Clearly, observing such changes strongly suggests that the well-being of the fish has been significantly compromised (reviewed in 32, 35, 36, 37).

To further complicate the assessment of stress in fish, recent studies have shown that chronic stress, including mild stress, can down-regulate (or deplete) the cortisol levels and/or compromise the normal stress response needed to deal with new challenges (8). A potentially more reliable measure of chronic stress is the pro-opiomelanocortin-derived (POMC-derived) peptide, a-melanocyte-stimulating hormone (a-MSH), released from the pituitary gland, that remains elevated in chronically stressed fish (33).

A current welfare concern in salmon aquaculture is the stress associated with the production of large numbers of smolts, the transitioning stage of the salmon as it matures from a freshwater juvenile into a marine-living adult. Successful season-independent intensive salmon production has become dependent on high throughput, reduced generation time, rapid development, high-energy diets, high densities, heated water and photoperiod control, with constant light to enhance growth and accelerate development. As freshwater resources are limited, increased demand for smolts is generally met by increasing the rearing densities, accompanied by a reduction in water use. Oxygen is routinely added to support production and seawater is frequently used to supplement freshwater resources, thus exposing the salmon to increased salinity, even at the earlier pre-smolt stages. There is increasing concern among producers, regulatory authorities and scientists that poor water quality may reduce smolt quality and subsequent postsmolt performance. Of particular concern are interactions between acidity, carbon dioxide and aluminium. Overall, a major challenge for salmon aquaculture is to ensure normal growth, development and welfare in a rearing environment that is increasingly intensive. Intensive farming methods can have direct physiological and health effects, and while these can have a substantial negative impact they are readily detectable. Chronic mild stress (CMS), however, is more difficult to diagnose in salmonids (38). In mammals, results show that CMS compromises coping ability and has long-term detrimental effects, especially for cognitive processes and affective states (39).

Effects of chronic mild stress

While transient mild stress can enhance learning and memory in mammals, chronic or severe stress disrupts hippocampus-dependent memory (reviewed in 40) and is linked to persistently high glucocorticoid levels. Hippocampal synaptic plasticity, as modelled by long-term potentiation, is widely believed to represent an important component mechanism of hippocampus-dependent memory formation. The effects of circulating glucocorticoids mirror that of stress: low levels amplify long-term potentiation (perhaps through preferential activation of hippocampal MR), while higher levels attenuate it (perhaps because they saturate the MR, leading to robust activation of GR [reviewed in 35]). Interestingly, in contrast to its latter effects on hippocampus-dependent learning, chronic stress can enhance amygdala (dorsomedial region in fish)-dependent learning, leading to enhanced fear learning and an increase in anxiety-related behaviours (see 41).

Stress and welfare

Previously, a homeostasis-based definition of welfare postulated a negative linear relationship between stress and welfare, where stability and no threats to homeostasis represent the best welfare. In recent years, a new concept of welfare based on allostasis suggests an inverted U-shaped relationship, where both too little or too much stress leads to poor welfare (42). The allostasis concept is an important model to discriminate between normal adaptive stress responses that are protective and situations that lead to poor animal welfare.

Good animal welfare is characterised by the capacity to anticipate and respond to environmental challenges in ways that match the environmental demands. Animals need the physiological capability, behavioural skills and cognitive abilities to predict and react to these demands appropriately (43, 44). Reducing these capacities leads to a mismatch between the response required by the particular conditions confronting the animal and the actual responses it mounts, thereby limiting its ability to experience good welfare. Understanding how the mediators of allostasis and coping ability are affected by stress level will give us a better understanding about the regulation of fish welfare.

Recent studies in fish have demonstrated that previous environmental experiences, whether negative (38) or positive (45), establish learning and memory-based mechanisms that enable the fish to more efficiently deal with environmental challenges. It is anticipated that these types of studies will provide new indicators for the evaluation of welfare and will aid in determining the stress thresholds that can provide optimal fish welfare and improve production.

Welfare on the fish farm in relation to pain and stress

There are multiple situations that may cause fish on farms to experience pain and unwanted stress, and several of these are considered by Huntingford and Kadri (46). Greater awareness of how routine procedures such as feeding, cleaning, handling and transportation can trigger stress responses or injuries in fish has led to changes in how these processes are undertaken (47, 48). However, other more subtle effects can impact on fish welfare, particularly if they influence the fish's cognitive and psychological processes (22). Changes in the timing of regular procedures, such as feeding times, have the potential to lead to frustration that may result in increased levels of aggression. Providing fish with more control of their environment (e.g. by installing self-feeders so that food can be available when the fish choose to forage as opposed to specific times of day) can help reduce problematic changes in behaviour (49).

That different species have different sensitivities to stressors is now more widely recognised; however, even within a species there is variation in how susceptible fish are to different kinds of stressor, or how sensitive they are to pain (49, 50, 51). Understanding how different coping styles influence fish stress physiology and behaviour is important, as some situations or environments may be acceptable to certain individual fish, but not to others (49). Recent work with fish has shown that the integrated behavioural and physiological mechanisms that comprise the distinct 'coping strategies' believed to be present in mammals are also evident in fish (52, 53).

Knowing the range of coping styles within a farm population could, therefore, be useful for informing farmers about which management practices to use or avoid.

Management of pain in terrestrial farm settings often requires the use of anaesthetics and/or analgesics. Studies of the effects of anaesthesia and analgesia in fish are only just beginning (54, 55). Morphine is a standard form of pain relief against which other potential analgesics are assessed, and morphine has been demonstrated to have analgesic effects in several species of fish (2, 56, 57). However, more research on fish anaesthetics and analgesics, in particular comparisons of different analgesic drugs and their effects on different species of fish, is needed.

An investigation with koi carp (*Cyprinus carpio*) contrasted the effects of saline (control) against ketoprofen (a non-steroidal anti-inflammatory analgesic) and butorphanol (an opioid analgesic) when an incision was made in the flank of the fish through to the body cavity – a procedure typically used for inserting internal telemetry tags (58). Butorphanol showed some analgesic benefit, in that the opercula beat rate and swimming behaviour of the carp treated with this drug was the same before and after surgery. A more recent experiment in rainbow trout investigated the effects of the opioid, buprenorphine, and a non-steroidal anti-inflammatory drug, carprofen (57). The buprenorphine had a negligible effect in terms of relieving pain associated with 0.1% acetic acid injection in the snout. The carprofen had more of an effect and fish treated with this drug resumed feeding more rapidly. Mettam *et al.* (55) also tested the effects of a local anaesthetic, lidocaine, applied to the snout at the same time as it was injected with saline or acetic acid (57). The result of the local anaesthetic was positive in that it helped to reduce several pain-related responses, for example, swimming activity and opercula beat rate.

Conclusions

Our ability to deliver good welfare for the fish we farm requires that we understand how they respond to stressful or noxious situations. This field is still relatively new compared to more established aspects of terrestrial farm animal welfare, and there is still considerable basic research that needs to be done, particularly in relation to the development of reliable, safe anaesthetics and analgesics. Studies directly assessing on-farm welfare of fish are currently demonstrating how relatively simple changes to traditional methods and practices can significantly improve both fish health and well-being. The authors believe that this area of research will develop rapidly over the next few years as more studies help to refine the techniques and methods used to farm different fish species.

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La douleur et les réactions de stress chez les poissons d'élevage

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Résumé

La pisciculture dont les produits sont destinés à l'alimentation humaine est un secteur en pleine expansion ; en raison des interactions devenues plus nombreuses avec les populations captives de poissons, on s'intéresse aujourd'hui davantage aux conditions qui permettent de protéger le bien-être de ces poissons. Les auteurs résument les avancées récentes des connaissances sur la douleur et sur les réactions de stress chez les poissons qui apportent un éclairage pertinent sur le bien-être des poissons d'élevage. Au cours des dix dernières années, la douleur chez les poissons a fait l'objet de plusieurs études, visant à établir son existence et, le cas échéant, à mesurer l'intensité de cette expérience aversive tout en recherchant des moyens de l'atténuer au moyen d'analgésiques. Il semble que les mécanismes neurologiques élémentaires assurant la détection d'une lésion tissulaire, à savoir les mécanismes nociceptifs, sont conservés des poissons aux oiseaux et aux mammifères ; toutefois, la question de l'intensité des affects négatifs associés à la douleur fait encore débat, ainsi que celle de savoir si les poissons y sont véritablement sensibles. Les réactions de stress, qui aident les poissons à faire face à diverses situations éprouvantes semblent également être conservées parmi l'ensemble des vertébrés et les changements physiologiques que manifestent les poissons suite à un stress aigu ou chronique ressemblent à ceux décrits chez les mammifères. Par conséquent, les poissons semblent dotés de la faculté innée d'éprouver des sensations négatives telles que la douleur et le stress, d'une manière analogue à celle que l'on retrouve chez d'autres vertébrés. Les situations potentiellement douloureuses ou stressantes pour les poissons d'élevage sont multiples ; la protection du bien-être de ces poissons exige donc de modifier les pratiques et les procédures appliquées en pisciculture. Il faut également tenir compte du fait que les besoins en termes de bien-être diffèrent selon les espèces élevées, certaines s'adaptant mieux que d'autres à l'environnement des fermes aquacoles. La thématique du bien-être des poissons est relativement récente et les travaux de recherche sur les réactions de stress, l'allostase, les seuils de douleur et les analgésiques doivent être poursuivis afin de contribuer à promouvoir le bien-être des poissons.

Mots-clés
Allostase – Bien-être – Douleur – Poisson d'élevage – Souffrance – Stress.

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Respuestas al dolor y el estrés en los peces de cultivo

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Resumen
La piscicultura con fines de consumo humano es un sector en auge permanente. Ahora, esta creciente interacción con poblaciones de peces cautivos se traduce en un interés cada vez mayor por la forma de generar estados positivos de bienestar en los peces que producimos. Los autores sintetizan lo que a día de hoy sabemos y entendemos sobre las respuestas de los peces al dolor y el estrés y la relación de estas reacciones con el bienestar de los peces de cultivo. En el curso del último decenio varios estudios se han detenido en la cuestión de si los peces sienten o no dolor, hasta qué punto una experiencia dolorosa genera aversión y de qué modo cabe paliarla con el uso de analgésicos. Aunque a grandes rasgos los mecanismos nociceptivos, esto es, los mecanismos neurales básicos que hacen posible la detección de daños tisulares, parecen haberse conservado en el tránsito evolutivo desde los peces hasta las aves y los mamíferos, hay controversia en torno al grado de emotividad negativa que acompaña al dolor y a si los peces la experimentan realmente o no. También la respuesta al estrés, que ayuda al animal a afrontar diversos problemas, parece haberse conservado en gran medida en los vertebrados, y los cambios fisiológicos que sufren los peces en reacción a situaciones de estrés agudo o crónico son similares a los descritos en los mamíferos. Los peces, por consiguiente, parecen dotados de la capacidad innata de experimentar estados negativos, como el dolor y el estrés, de un modo análogo a como los viven otros vertebrados. Hay numerosas situaciones en que los peces de cultivo pueden experimentar dolor y estrés, y cada vez está más claro que para hacer posible un estado conveniente de bienestar será preciso modificar las prácticas y procedimientos de la piscicultura. También es un hecho

que las necesidades de bienestar de las diferentes especies piscícolas no son necesariamente iguales, y que algunas especies sobrellevan mejor que otras las condiciones propias del cultivo en cautividad. Dado que el tema del bienestar de los peces es relativamente nuevo, para ayudar a promover estados positivos de bienestar en los peces se precisan más investigaciones sobre respuestas al estrés, alostasis, umbrales de dolor y analgésicos.

Palabras clave
Alostasis – Bienestar – Dolor – Estrés – Peces de cultivo – Sufrimiento.

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