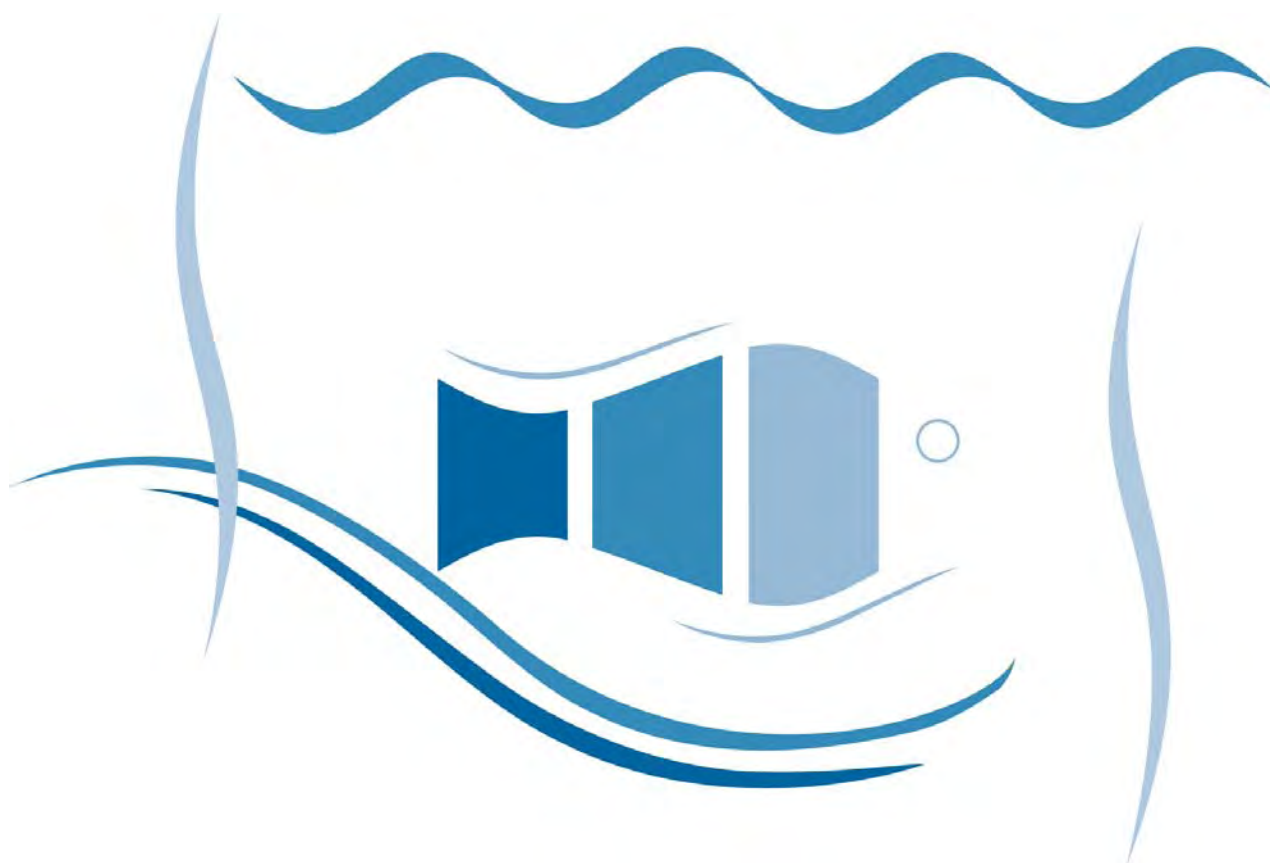


COST 867: International Workshop on Fish Welfare

8th– 10th February 2011
Madrid, Spain



International Workshop on Fish Welfare: Cost 867 Final Meeting
February 8-10, 2011, Madrid Spain
Local Organiser: Morris Villarroel
Departamento de Producción Animal
Escuela Técnica Superior de Ingenieros Agrónomos
Universidad Politécnica de Madrid
C/ Ciudad Universitaria s/n
28040 Madrid
morris.villarroel@upm.es

COST 867: International Workshop on Fish Welfare

8th – 10th February 2011

***Escuela Técnica Superior de Ingenieros Agrónomos,
Madrid, Spain***

TUESDAY, FEBRUARY 8th (Cost 867 delegates only)

15:00 – 16:00	Special issue
16:00 – 17:00	Joint meeting WGs
17:00 – 18:00	MC meeting

WEDNESDAY, FEBRUARY 9th

Welcome Address and Keynote Lectures

Anders Kiessling, Norwegian University of Life Sciences, Norway

09:00 – 09:45	Dan Weary , University of British Columbia, Canada <i>A Canadian's perspective on the changing face of animal welfare: what's hot, what's not, and what to expect in the coming years</i>
09:45 – 10:30	Victoria Braithwaite , Penn State University, USA <i>Fish pain and suffering - current understanding and future directions</i>
10:30 – 11:00	Coffee break

SESSION 1

Chair: **Børge Damsgard**, NOFIMA, Norway

11:00 – 11:30	Neill Herbert , University of Auckland, New Zealand <i>A role for exercise in the welfare of farmed fish?</i>
11:30 – 11:45	Catarina Martins , University of Algarve, Portugal <i>Short talk: Behavioural indicators of farmed fish welfare</i>

11:45 – 12:15 **Ryan Wilkinson, University of Tasmania, Australia**
Aggressive behaviour and cannibalism in juvenile barramundi, implications

12:15 – 12:45 **Danielle Champagne, Leiden/Amsterdam Center for Drug Research**
The usefulness of the zebrafish model to understand the impact of environmental stress on health

12:45 – 13:00 Discussion

13:00 – 14:30 **Lunch**

Keynote Lecture

14:30 – 15:00 **John Purser, University of Tasmania, Australia**
Challenges in the salmon industry with a focus on climate change

SESSION 2

Chair: **Ana Roque, IRTA, Spain**

15:00 – 15:15 **Øyvind Øverli, Norwegian University of Life Sciences, Norway**
Neuroendocrine aspects of welfare research in teleost fish: Current approaches

15:15 – 15:30 **Anders Kiessling, Norwegian University of Life Sciences, Norway**
Physiological stress response in rainbow trout to graded levels of CO₂ and temperature changes

15:30 – 15:45 **Cedric Mathieu, University of Namur, Belgium**
Do gluco- or mineralocorticoids regulate immunity of Eurasian perch?

15:45 – 16:00 **Javier Sánchez, University of Murcia, Spain**
Environmental cycles and welfare of fish larvae

16:00 – 16:30 **Coffee break**

16:30 – 16:45 **Leonor Galhardo, Instituto Superior de Psicologia Aplicada, Portugal**
Use of a push-door to measure motivation in cichlids

16:45 – 17:00 **Hans van de Vis, University of Wageningen, Netherlands**
Slaughter of fish using ice, ECG data

17:00 – 17:15 **Rui F Oliveira, Instituto Gulbenkian de Ciencia, Portugal**
Cognitive appraisal is needed to trigger a physiological response to a challenge: implications for fish welfare

17:15 – 18:30 **Poster sessions**

THURSDAY, FEBRUARY 10th

Keynote Lectures

Morris Villarroel, Universidad Politécnica de Madrid, Spain

09:00 – 09:45 **Xavier Manteca**, Universidad Autónoma de Barcelona, Spain
Welfare assessment in fish: can we learn from the Welfare Quality experience?

09:45 – 10:30 **Bernice Bovenkerk**, Ethics Institute, Utrecht, Netherlands
The interplay between science and ethics

10:30 – 11:00 **Coffee break**

SESSION 3

Chairs: **Neil Duncan**, IRTA, Spain; **Alan Dykes**, Scottish Salmon Company

11:15 – 11:45 **Tomasz Pinkiewicz**, University of Tasmania, Australia
A computer vision system to analyse the swimming behaviour of sea-caged salmon

11:45 – 12:00 **Tore S. Kristiansen**, Institute of Marine Research, Norway
Short talk: SWIM- salmon welfare index model

12:00 – 12:15 **Pierluigi Carbonara**, COISPA Tecnologia & Ricerca, Italy
Short talk: Multiparametric approach to assess welfare in sea bass organic aquaculture

12:15 – 12:45 **Rasmus Kaspersson**, University of Gothenburg, Sweden
Smoltpro: sustainable smolt production, an integrated approach

13:00 – 14:30 **Lunch**

Keynote Lecture

14:30 – 15:15 **Peter Sandøe**, Danish Centre for Bioethics and Risk Assessment, University of Copenhagen, Denmark
The changing agenda of animal ethics, how do fish fit in?

Session 4

Chair: **Sunil Kadri**, AqualInnovation, United Kingdom

15:15 – 15:30 **Michael Appleby**, World Society for the Protection of Animals & Farm Animal Welfare Committee, UK
Fish welfare: a British and worldwide perspective

15:30 – 15:45 **Javier Ojeda**, APROMAR, Spain
Mediterranean farmed fish also have rights

15:45 – 16:15	Bob Waller , Freedom Food, United Kingdom John Aviezhinius , RSPCA, United Kingdom <i>Freedom food and aquaculture: 10 years on</i>
16:15 – 16:45	Coffee break
16:45 – 17:00	Adriaan Kole , University of Wageningen, Netherlands <i>Willingness to pay and possible development of the market for welfare products</i>
17:00 – 17:30	Anders Kiessling , Norwegian University of Life Sciences, Norway <i>What's next?</i>
17:30	Closing

Location

Salón de Actos

Escuela Técnica Superior de Ingenieros Agrónomos (Agricultural Engineering School)

Universidad Politécnica de Madrid, Ciudad Universitaria, 28040, Madrid

<http://www.etsia.upm.es/ETSIAgronomos/Escuela/Instalaciones/Localizacion>

Sponsors

COST (www.cost.esf.org)



Escuela Técnica Superior de Ingenieros Agrónomos



Universidad Politécnica de Madrid (UPM)



POLITÉCNICA

Sociedad Española de Acuicultura (SEA)



Instituto Nacional de Investigaciones Agrarias (INIA)



Fundación Observatorio Español de Acuicultura (FOESA)



Comunidad de Madrid (CAM-Newgan)



COST 867: International Workshop on Fish Welfare

Chair: **Børge Damsgard**, NOFIMA, Norway

WEDNESDAY, FEBRUARY 9th

9:00-13:00

Session 1.



A Canadian's perspective on the changing face of animal welfare: what's hot, what's not, and what to expect in the coming years

Dan M. Weary,

Animal Welfare Program, The University of British Columbia, 2357 Main Mall, Vancouver, BC, V6T 1Z4, Canada

dan.weary@ubc.ca

The field of animal welfare science has advanced rapidly in recent years. The aims of my presentation will be to highlight concepts and methodology that are changing what is meant by 'welfare' and how it is assessed. Much attention has focused on direct harms to the animal associated with methods of care and use, such as pain and distress at the time of slaughter, welfare is increasingly viewed as more than the lack of harms and instead on providing animals a 'good life'. Anticipatory behavior testing and studies of cognitive bias can be providing new insights into how animals evaluate their welfare, and how living conditions can be improved. Methods of preference testing continue to provide an important window into what environmental features are important to animals, and the study of environmental enrichment is shifting from simple additions to impoverished environments to changes that stimulate animals via exploration, play and the provision of positive contrasts. In the coming years the unintended consequence of production practices on animal welfare will likely come under increased scrutiny, strengthening links in the public's mind between welfare and ecological impact, and spawning new research on welfare and conservation. These changes highlight the democratization of welfare, with the agenda increasingly defined by public versus expert values, inspiring new research on public attitudes to animal welfare. Research on public attitudes can provide a real-time "Turing test" of a good life, and for identifying and correcting contentious practices.

Fish pain and suffering - current understanding and future directions

Victoria A. Braithwaite

Penn State University, USA

V.Braithwaite@psu.edu

There is now considerable evidence that fish respond to noxious or harmful situations in ways that are more than just a reflex response. Studies have revealed how nerve fibers convey information about a damaging stimulus from the spinal cord and brain stem to areas within the forebrain. Determining how this information is processed and perceived has been more difficult, but again experiments have shown how higher order cognitive processes are disrupted by noxious stimulation indicating that the fish may be aware of their pain and discomfort. Importantly, impaired cognitive responses can be overcome if pain relieving drugs are used. Current research suggests that fish are sentient creatures that are capable of experiencing negative feelings associated with pain. The way we house, handle and interact with fish, particularly in the production setting, has the capacity to trigger both fear and suffering. Finding ways to minimize this will be beneficial to both the fish and those that culture fish. This presentation reviews our current understanding of fish pain perception and considers what kinds of information we need to help improve the welfare of the fish we rear through aquaculture.

A role for exercise in the welfare of farmed fish?

Neill A. Herbert

Leigh Marine Laboratory, University of Auckland, PO Box 349, Warkworth 0941, New Zealand

n.herbert@auckland.ac.nz

Sustained levels of moderate swimming improves the growth and feed conversion efficiency of several farmed fish species but relatively little is known about the welfare benefits of exercise. I will therefore review the potential welfare benefits of exercise by considering the following set of interrelated questions: 1) Can induced-exercise support welfare through expression of “natural” swimming behaviour in aquaculture? 3) Is there a difference between voluntary and forced exercise? 4) Are there any negative aspects of sustained exercise that are likely to compromise welfare? 5) How important is exercise to the welfare of different farmed species? This presentation is based on published literature and work carried out by the author on a range of different species.

Social network analysis of behavioural interactions leading to fin damage in Atlantic salmon parr (*Salmo salar*) during feed-restriction

Hernán Alberto Cañon Jones¹, Linda A. Hansen², Chris Noble², Børge Damsgård², Donald M. Broom¹ and Gareth P. Pearce¹.

¹: *Department of Veterinary Medicine, University of Cambridge, United Kingdom.* ²: *Nofima Marin, Tromsø, Norway.*

hac39@cam.ac.uk

Social network analysis of the behavioural interactions amongst Atlantic salmon (*Salmo salar*) was used to quantify the effect of feed restriction in the development of fin damage. Dorsal fin damage and erosion was present only in groups subjected to feed-restriction and it was positively correlated with aggression and fin-biting. Also, fish under feed restriction had significantly lower weight gain, reduced growth-rate and body condition but there was no difference in total length compared to control groups. Social networks analysis of aggressive interactions (bites, attacks and displacements) in feed restriction groups showed a significantly lower distance, but a higher density, higher clustering coefficient and higher in (amount of aggression received) and out (amount of aggression initiated) degree centrality. These findings indicated a distinctive separation of roles according to aggression only found in feed-restriction groups. Initiators of aggression had high out-degree centrality, less fin damage, gained more weight and attained more central positions within the school of fish. Receivers of aggression had high in-degree centrality, more fin damage and gained less weight. Social network analysis of associative behaviour showed significantly lower values in transitivity and distance with a tendency for higher centrality in the feed-restricted groups indicating an imbalance in their social relationships and ties. The present study demonstrated the value of social network analysis in investigating behavioural interactions associated with aggression and the development of fin damage in Atlantic salmon.

Behavioural indicators of farmed fish welfare

Catarina I M Martins^{1,2*}, Leonor Galhardo³, Chris Noble⁴, Børge Damsgård⁴, Maria T. Spedicato⁵, Walter Zupa⁵, Marilyn Beauchaud⁶, Ewa Kulczykowska⁷, Jean-Charles Massabuau⁸, Toby Carter⁹, Sònia Rey Planellas¹⁰ and Tore Kristiansen¹¹

¹CCMAR - CIMAR L.A., Centro de Ciências do Mar do Algarve, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal, ² Aquaculture and Fisheries Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands, ³ Unidade de Investigação em Eco-Etologia, Instituto Superior de Psicologia Aplicada, Rua Jardim do Tabaco 34, 1149-041 Lisboa, Portugal, ⁴ NOFIMA marine, P.O. Box 6122, 9291 Tromsø, Norway, ⁵ COISPA Tecnologia & Ricerca, via dei Trulli, 18-20 – 70126 Bari, Italy, ⁶ Laboratoire Ecologie et Neuro-Ethologie Sensorielles (EA3988), Université Jean Monnet, 23 rue Dr Paul Michelon, 42023 Saint-Etienne Cedex 02, France., ⁷ Institute of Oceanology, Polish Academy of Sciences, 81-712 Sopot, Poland, ⁸ Station Marine. Université Bordeaux 1, CNRS, UMR 5805 EPOC, Place du Dr Peyneau, 33120, Arcachon, France, ⁹ Anglia Ruskin University, East Road, Cambridge, CB1 1PT, UK, ¹⁰ Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallés, Barcelona, Spain ¹¹ Institute of Marine Research, P.O. Box 1870, N-5817 Bergen, Norway, cimartins@ualg.pt

Behaviour represents the reaction to the environment as fish perceive it and therefore is a key element of fish welfare. This review summarises the main findings on how behavioural changes have been used to assess welfare, on its both functional and feelings-based approaches, in farmed fish. Changes in foraging behaviour, ventilatory activity, aggression, individual and group swimming behaviour, stereotypic and abnormal behaviour have been linked with acute and chronic stressors in aquaculture and therefore can be used as indicators of poor welfare. On the contrary, the measurement of exploratory behaviour, feed anticipatory activity and reward-related operant behaviour are starting to be considered as indicators of positive emotions and welfare in fish. The willingness to accept that fish are sentient creatures capable of positive and negative emotions may contribute to the development of new strategies (e.g. environmental enrichment) to foster good welfare. Lastly, the published studies using behavioural indicators of welfare show that behavioural changes can be interpreted as either good or poor welfare depending on the fish species. Therefore it is essential to understand the species-specific biology before drawing any conclusions in relation to welfare. In addition, different individuals within the same species may exhibit divergent coping strategies towards stressors and what is tolerated by some individuals may be detrimental to others. Therefore the assessment of welfare in a few individuals may not represent the average welfare of a group and vice versa. This underlines the need to develop on-farm, behavioural welfare indicators that can be easily used to assess not only the individual welfare but also the welfare of the whole group (e.g. spatial distribution). With the ongoing development of video technology and imaging processing the on-farm surveillance of behaviour may in the near-future represent a cheap, non-invasive tool to assess the welfare of farmed fish.

Aggressive behaviour and cannibalism in juvenile barramundi (*lates calcarifer*) – implications for commercial culture

Ryan Wilkinson, Timothy Digory Hulse, John Purser

National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Locked Bag 1370, Launceston, Tasmania, Australia, 7250

Ryan.Wilkinson@utas.edu.au

Barramundi culture commenced in Australia in the mid 1980's and since then production has increased steadily. Barramundi has recently been listed as one of the fastest growing sectors in worldwide aquaculture and is an important source of employment in rural and regional Australia. One major problem in the industry is the high rate of aggressive interactions (i.e. chasing, biting, fin-nipping) and cannibalism which is observed during the larval and juvenile phase of culture. In some instances, if left unmanaged, this cannibalism may result in up to 20-50% mortality of the tank population. To combat this problem frequent management intervention (i.e. size-grading of individuals) is required. This labour-intensive process occurs up to 3 times per week throughout the nursery phase. Although a necessary process, these grading operations are costly and are stressful for the fish. Outbreaks of disease can often be linked back to poor husbandry during grading operations. Thus, aggression and cannibalism in barramundi and the resultant frequent handling and grading of fish are all seen as potential welfare issues in commercial culture. This presentation will discuss the current status of barramundi farming in Australia and summarise our preliminary attempts to better understand behaviour and aggression in this fish species. Results from studies involving video monitoring of behaviour, body damage scoring, physiological stress indicators, photoperiod manipulation, feeding frequency and fluvariums (i.e. group recognition) will be presented. In addition, future research directions investigating the potential for individual relatedness and/or familiarity to influence aggression and cannibalism in barramundi will also be discussed.

The usefulness of the zebrafish model to understand the impact of environmental stress on health

Danielle Champagne

Department of Integrative Zoology, Institute of Biology of Leiden, University of Leiden, The Netherlands.

d.champagne@chem.leidenuniv.nl

The zebrafish model is becoming increasingly used in stress research. We have recently developed and characterized behavior-based assays allowing measurements of complex phenotypes of brain function including stress, anxiety, and cognition. The modeling of these phenotypes is not only highly relevant but is a requisite in the fields of psychiatry, neuropharmacology, preclinical drug screening research, and for assessment of the welfare of animals in laboratories, farms, and aquaculture industries. Specifically, the open field and light/dark preference tests are commonly used for these purposes and have been successfully adapted from rodent to (adult and larval) zebrafish models. We recently showed that both adult and larval zebrafish express a range of stress/anxiety-like behaviors comparable to those observed in other species including humans. Pharmacological treatments with commonly used anxiolytic and anxiogenic drugs confirmed the 'anxiety-like' nature of these behaviors. We used these behavioral assays to reveal the lasting impact of stressful environments during development as well as during acute exposure to stress in later life. We report that high level of stress hormone (cortisol) and inappropriate level of social interactions during critical periods of development (embryogenesis) lead to lasting alterations in the stress-regulating system, which render fish more vulnerable to stressful challenges later in life. These findings are relevant in the light of the large body of evidence linking vulnerability to immune diseases/infections and inappropriate stress coping/response. Taken together, these findings show the feasibility of transferring traditional rodent methodology to zebrafish as well as the usefulness of the zebrafish model and its methodology to routinely assess fish welfare in laboratories, farms, and aquaculture industries throughout the lifespan of the fish.

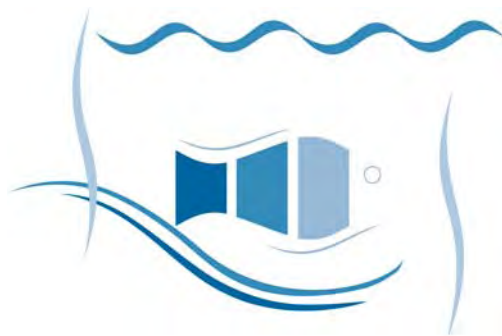
COST 867: International Workshop on Fish Welfare

Chair: **Ana Roque**, IRTA, Spain

WEDNESDAY, FEBRUARY 9th

14:30-17:30

Session 2.



Challenges in the Tasmanian salmon industry with a focus on climate change

John Purser¹, Stephen Battaglene², Chris Carter², Alistair Hobday³, Robin Katersky¹, Vincent Lyne³, Barbara Nowak¹, Tomasz Pinkiewicz¹, Ryan Wilkinson¹

¹*National Centre for Marine Conservation and Resource Sustainability, AMC, University of Tasmania, Launceston, Tasmania, Australia,* ²*Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia,* ³*Marine Climate Impacts and Adaptation, CSIRO, Hobart, Tasmania, Australia*
jpurser@amc.edu.au

With an annual production of c. 35,000 tonne (value of c. \$350m), the Tasmanian salmon industry is the largest aquaculture sector in Australia contributing significantly to the Tasmanian state economy. In world terms it is relatively small but produces a diverse range of high quality products for principally the domestic market. The industry is a rapidly expanding sector (started in 1985) currently in the process of securing more coastal water for leases to facilitate this expansion. The industry is located in the southern-most state of Australia between latitudes 41-43.5oS. Temperatures are at the upper end of the range for Atlantic salmon, rainbow trout and brook trout, producing high growth rates and high levels of early maturity, with most fish harvested after one sea winter or 15-20 months under cage production. However, there are fears that any further increases in temperature may magnify existing problems and introduce new ones. Particular issues have challenged the industry. These have included world-related issues such as fish oil and meal shortages, marketing issues such as year-round supply, maturation management, harvesting and fish quality, and local issues such as Amoebic Gill Disease and other health factors, feeding, nutrition, seals, jellyfish, 'pinheading', environmental impacts, invasive species, algal blooms and sea water temperatures. Sea water temperatures are normally influenced by the East Australian Current from the north, the Tasman Sea circulation from the east and the Antarctic Circumpolar circulation to the south with the industry concerned about the potential effects of increases in sea water temperature as a consequence of global warming. This talk will detail the issues faced by the industry with a particular focus on how recent warm summers and potentially increasing water temperatures due to climate change may impact salmon production. Initiatives developed by the industry also will be outlined.

Physiological stress response in rainbow trout to graded levels of CO2 and temperature changes

Aleksandar Vidakovic*, Brankica Djordjevic*, Henrik Seth*, Erik Sandblom*, Michael Axelsson, Anders Kiessling.*All contributed equally in these studies.

Norwegian University of Life Sciences

anders.kiessling@vfm.slu.se

Aquaculture involves several procedures, as transport, pumping, sedation etc. where metabolic or externally added CO2 result in a hyper capnia. Also graded changes in temperature occur as a result of different handling procedure or occur naturally during farming. The welfare of the fish exposed to such changes has been debated. The main problem investigating the physiological stress response, as a measure of reaction to such changes, is the sampling itself. Netting or reduction of water level etc. all will result in a stress response possible masking the effect of the treatment. Another problem with terminal sampling is of course that it only represents an accumulated value up to sampling. Collecting data from free swimming fish, only affected by treatment offers the possibility to obtain data representative of the actual treatment as well as the profile of response over time. In this paper we will present data obtained from free swimming rainbow trout and Arctic charr fitted with DA-cannula and exposed to graded levels of CO2 and temperature. We will also discuss the implication of the data and how we will proceed with this work in order to secure that these data are representative also of the practical situation.

Does gluco- or mineralocorticoid regulate immunity of eurasian perch?

Cédric Mathieu, Sylvain Milla, Robert Mandiki, Jessica Dourfils, Patrick Kestemont

University of Namur (FUNDP), Department of Biology – URBO, Rue de Bruxelles, 61, 5000 Namur, Belgium

cedric.mathieu@fundp.ac.be

In fish, cortisol has been highlighted as the main welfare/stress indicator during previous COST 867 meeting. Cortisol is the main corticosteroids in fish and it can bind to both gluco- (GR) and mineralocorticoid receptors (MR). In a large diversity of fish species it has already been described that cortisol regulates various physiological systems such as immunity, glucose metabolism and hydromineral balance. In mammals mineral balance is regulated by aldosterone and this hormone has also been shown to regulate some immune parameters. Recently 11-deoxycorticosterone (DOC) has been highlighted to bind MR and maybe counteract the lack of aldosterone in fish. But the DOC functions are not described in fish yet. We have investigated the modulation of the Eurasian perch immune system by these two corticosteroids injected intraperitoneally. During this study we have shown that cortisol, but also DOC, affect the fish immune status. Cortisol changed the leucocytes proportion in blood circulation. Lymphocyte proportion drastically decreased in favour of neutrophil proportion. Cortisol and DOC also decreased lysozyme activity in the blood 6h after the injection. Moreover, in the gills, cortisol and DOC regulated the expression of several genes coding for proteins implicated in the immune response (cytokine or complement C3 for example). All these regulations suggest that these two corticosteroids regulate fish immunity. This regulation seems to promote innate immunity. Their action seems to be mediated by the corticosteroid receptor. Actually we also observed an increase in the expression of genes coding for GR-2 and MR in the gills of fish injected with cortisol.

Environmental cycles and welfare of fish larvae

Javier Sánchez

University of Murcia, Spain

Light and temperature cycles are key environmental factors that synchronize rhythms at all life-stages of fish, from embryo development to sexual maturation. On one hand, the underwater photo-environment is complex since light characteristics (i.e. intensity, photoperiod and spectrum) depend on the absorbance properties of the water column, so that blue wavelengths become predominant with depth. However, standard lighting systems (bulbs and fluorescent lamps) commonly used in hatcheries are shifted towards the red wavelengths and are kept continuously on (LL) or off (DD), light conditions that are not environment-specific and could compromise fish welfare and provoke skeletal abnormalities. On the other hand, the cyclic infrared radiation from the Sun generates a thermocycle (TC): during the day the temperature rises (thermophase), while during the night the temperature drops (cryophase). Temperature has long been reported to influence growth and development of fish larvae both in the wild and laboratory, but little is known about the effects of a daily TC. Recent research pointed to dramatic changes in larvae growth performance, survival, development and sex ratio, caused by exposure to different temperature regimes (constant vs TC). The aim of this review is to look at the effects of environmental cycles (LD and TC) on fish larvae welfare of commercial interest in aquaculture, discussing the artificial rearing conditions used by fish farmers to maximise fish survival and growth.

The use of a push-door for measuring motivation in a cichlid fish

Leonor Galhardo^{1*}, Olinda Almeida¹ & Rui F. Oliveira^{1,2}

¹ *Unidade de Investigação em Eco-Etologia; Instituto Superior de Psicologia Aplicada, Rua Jardim do Tabaco, 34 · 1149-041 LISBOA · PORTUGAL*, ² Champalimaud Neuroscience Programme; Instituto Gulbenkian de Ciencia, Rua da Quinta Grande, 6 · 2780-156 Oeiras · Portugal
leonor_galhardo@ispa.pt

Recent research suggests that fish are capable of mental experiences. Therefore, identifying needs from the animals' point of view is likely to be one of the best approaches to understand their specific needs. Motivational tests, as a measure of how animals value certain resources, have been developed and refined for some decades. The aim of this study was to adapt for the first time a push-door to quantify motivation in a cichlid fish, the Mozambique tilapia (*Oreochromis mossambicus*). Males of this species have strong snouts which they use for a number of activities and thus they are suited to push. Twelve males of different social status were tested for three kinds of reinforcers: food, social partner and a control (additional space with substrate only). The animals were required to work at the door (push/touch) at an ascending cost in order to have access to the resources. Measures of motivation included latency to open the door, work attention and maximum price paid. Latency to open the door increased with increasing cost for all resources, with the highest latency for the control reinforcer. Work attention was constant with increasing cost for social partner and food, and always higher than the control. Maximum price paid was consistent with these results, being higher for social partner and food than for the control. The results of the three measures were consistent with each other and showed that the push-door can be used to measure motivation in this species.

Cognitive appraisal is needed to trigger a physiological response to a challenge: implications for fish welfare

RF Oliveira, JM Simões, AC Oliveira

ISPA-Instituto Universitário, Lisboa, Portugal, Champalimaud Neuroscience Program, Instituto Gulbenkian de Ciência, Oeiras, Portugal

Challenges in the life of animals (e.g. stressors) elicit physiological responses that prepare the organism to deal with emergency situations. The appraisal theory proposes that responses to challenges arise, not just as a result of direct perceptual information, but also depending on an evaluation of the meaning of that perceptual information to the organism at that moment in time. Thus, during the appraisal process a cognitive dimension differentiates how the animal experiences the challenge beyond mere valence (positive vs. negative). In this experiment we used mirror elicited aggression in zebrafish to create fights where the appraisal of the outcome (winning vs. losing) is not available to the subject and compared these to real opponent fights where appraisal of winning/ losing is available, in terms of changes in brain transcriptome. The prediction is that if appraisal is needed to elicit responses in neurogenomic states, then mirror fights should not elicit the same patterns of changes in gene expression in the brain as those observed in real opponent fights. This prediction is confirmed by our results: the number of differentially expressed genes in the brain of fighting zebrafish is higher in losers than in winners and it is zero in mirror fighters. Socially regulated genes in winners and losers include immediate early genes (e.g. c-fos) and genes involved in neural plasticity (e.g. MAPK pathway, including BDNF). Together these results suggest that social interactions drive short term changes in neurogenomic states that underly neural plasticity and affect subsequent behavior, and that these depend not on the expression of fighting behavior per se but on the appraisal of the outcome of the contest. Thus, physiological responses to social challenge in zebrafish are modulated by cognitive appraisal. The occurrence of appraisal in fish implies that responses to challenges are not mere reflexes and that a cognitive dimension can modulate these responses.

COST 867: International Workshop on Fish Welfare

Applied assessment of fish welfare

Chairs: **Neil Duncan**, IRTA, Spain
Alan Dykes, Scottish Salmon Company

THURSDAY, FEBRUARY 10th

09:00-14:30

Session 3.



Welfare assessment in fish: can we learn from the Welfare Quality® experience?

Xavier Manteca

Universidad Autónoma de Barcelona, Spain

The Welfare Quality® project has developed a system to enable overall assessment of welfare of cattle, pigs and chickens. The protocols follow a top-down approach: four main welfare principles were identified, they were split into twelve independent welfare criteria and finally measures were selected to assess these welfare criteria. The four welfare principles were (1) are the animals properly fed, (2) are the animals properly housed, (3) are the animals healthy, and (4) does the behaviour of the animals reflect optimized emotional states?. Welfare Quality® has based its welfare assessment essentially on animal-based measures and whenever possible these have been evaluated with respect to their validity, reliability and feasibility. The objective of this paper is to discuss whether the conceptual and methodological approach of Welfare Quality® can be applied to assess welfare in fish.

Expert meeting Fish Welfare: the interplay between science and ethics

Bernice Bovenkerk, Franck Meijboom

Ethics Institute, Heidelberglaan 8, 3584 CS Utrecht, Netherlands

b.bovenkerk@uu.nl

On November 29 and 30, 2010, the Ethics Institute of Utrecht University organised an expert meeting about fish welfare and its moral implications. This expert meeting brought together international experts from marine biology, physiology, the philosophy of mind, and ethics. The first day focused on the question what we can learn from research into fish emotion, cognition, and awareness. No consensus exists between biologists about strategies and outcomes in fish research. Some argued that fish do not consciously experience emotions, by looking at two signs of emotion – increased heartrate and temperature – and showing that these were not affected in fish after negative or positive stimuli. Others argued that these strategies did not take into account the special physical make-up of fish and showed that we can learn a lot from looking at other parameters. While stressing the enormous variation between different kinds of fish, they argued that an examination of the central nervous system and behaviour of fish gives us indirect evidence for cognition and emotion in many fish. On day two the results from the first day were related to current thinking in the field of animal ethics. It became clear that different theoretical viewpoints frame the question whether fish have moral status quite differently. Nonetheless, surprising consensus appeared to exist between different theories that at least some animals are part of our moral community. However, on the specific question whether fish should be included in this group, the experts were still unclear. More input of empirical research is needed, but as empirical research is not value-neutral more interaction should take place between empirical scientists and ethicists.

A computer vision system to analyse the swimming behaviour of seacaged salmon *Salmo salar*

Tomasz Pinkiewicz^a and John Purser^b

^a*School of Computing and Information Systems, University of Tasmania, Locked Bag 1359, Launceston, Tasmania 7250, Australia*, ^bNational Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Locked Bag 1370, Launceston 7250, Tasmania 7250, Australia
TomaszP@utas.edu.au

Knowledge of fish behaviour plays an important role in aquaculture farm management. A video system is the most common and cost-effective way in sea cages of observing behaviours during feeding and on an ad hoc basis. However longer-term observations are not possible due to operator fatigue and a limited ability to analyse video footage. This presentation introduces a computer vision system that was developed to automatically detect fish images on video footage and from that analyse fish speed and movement. Experiments were carried out on Atlantic salmon (*Salmo salar*) smolt where the objective was to detect when the schooling behaviour commenced following transfer to a sea-cage from the hatchery. Results show that the system is capable of detecting changes in speed and direction continuously throughout the day and there was a clear and quite sudden shift from non-schooling to schooling behaviour about 30 days post-transfer of the smolt. Also variations in diurnal swimming speed and directional patterns were detected and these may have been associated with the daily shift in the tidal cycle. Results from the smolt transfers, feeding, and husbandry activities together with potential applications to environmental change and seal attack will be presented. The system has the potential to provide useful information about fish welfare to farm operators on a continuous, real-time basis.

SWIM – Salmon welfare index model

T. S. Kristiansen¹, L. H. Stien^{*1}, M. Bracke², B., O. Folkedal¹, A. Kiessling, S. Kittelsen⁴, P. J. Midtlyng⁴, F. Oppedal¹, Ø. Øverli³

¹*Institute of Marine Research, 5392 Storebø, Norway*. ²Wageningen University and Research Centre, P.O. Box 65, 8200 AB Lelystad, The Netherlands, ³Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway, ⁴Norwegian School of Veterinary Science, PO Box 8146 Dep, 0033 Oslo, Norway
lars.stien@imr.no

The authorities and stakeholders are strongly asking for scientific support to develop science-based tools and protocols for fish welfare assessment. To address this need, we will construct a semantic model and tool for overall welfare assessment of farmed Atlantic salmon (*Salmo salar* L.). By overall assessment of welfare (OWA) we mean a systematic attempt to assess the welfare status of animals based on observations of the animals, their biological and physical environments, and available scientific knowledge. In semantic modelling (SM) welfare is defined as the quality of life as perceived by the animals themselves and both positive and negative aspect of welfare are considered. SM has been designed for the purpose of formalized assessment of animal welfare based on available scientific information, including scientific knowledge and scientific descriptions of housing systems in terms of both environment-based and animal-based measures. The first step of welfare assessment by semantic modelling approach is to collect a list of the species' basic needs, and to collect a list of scientific statements, obtained from a systematic literature review using the criterion that the statements are somehow relevant to assess welfare in aquaculture. The second step is to create a list of measurable or observable attributes ("animal-based and environmental welfare indicators") from the scientific statements that can be linked to at least one need, to ensure that all attributes in the model are relevant to welfare from the animal's point of view. The further process and challenges will be explained and discussed. See also www.imr.no/salmowa.

Multiparametric approach to assess welfare in sea bass organic aquaculture

Pierluigi Carbonara^a, Ilaria Corsi^b, Silvano Focardi^b, Robert Scott McKinley^c, Maria Scolamacchia^{ad}, Walter Zupa^a, Maria Teresa Spedicato^a, Giuseppe Lembo^a

^a *COISPA Tecnologia & Ricerca, stazione sperimentale per lo studio delle risorse del mare*, ^b Department of Environmental Sciences "G. Sarfatti", University of Siena, ^c Centre for Aquaculture and Environmental Research, University of British Columbia, Vancouver, ^d Department of General and Environmental Physiology, University of Bari
carbonara@coispa.it

Three groups of sea bass were fed for seven months with three different diets. The first was a conventional one, while the other two organic diets differed from the first one for the presence of organic vegetable compounds, a greater content in vegetable proteins and for the presence of a natural antioxidant compound (essential oil of rosemary). The two organic diets differed one from each other for their content in crude proteins and total lipids. In this study, the relationship between these three diets and sea bass welfare was investigated through the use of 16 different validated welfare indicators. Physiological indicators linked with the swimming activity as *recovery test* and the remote control of the muscle activity such as electromyograms, (EMG); haematological descriptors of the stress: haematocrit, haemoglobin, red blood cell count, cortisol, glucose, lactate; aspecific immunity: lysozyme; exposure to organic contaminants: EROD and GST; zoo-technical parameters: weight gain SGR, FCR, PER and HSI. Most of these singular parameters gave coherent responses, but only their integration in a comprehensive diagnostic frame allowed defining different welfare conditions of the three experimental groups. Both the swimming performances (*recovery test*) and the red muscular activity (EMG) represented the whole organism response to the different treatments. They could be suggested as synthetic and qualitative descriptors of both physiological and metabolic dynamics in reared sea bass. The overall best welfare condition was achieved in fish fed with the organic diet richer in protein.

SMOLTPRO: sustainable smolt production, an integrated approach

Rasmus Kaspersson, Jörgen I. Johnsson

Department of Zoology, Animal ecology, University of Gothenburg, Box 463, SE-405 30 Göteborg, Sweden.
rasmus.kaspersson@zool.gu.se

Anadromous salmon and trout are important natural resources for recreation and fishing, and are a part of our cultural heritage. Human activities, however, have reduced the natural production of salmonids considerably. In an attempt to partly compensate for this there have been large releases of hatchery-produced salmon and trout for decades, yet only few have been recaptured and in recent years, the numbers have declined dramatically. SMOLTPRO (launched in January 2010) is a four-year strategic project funded by the Swedish Research Council Formas. The main aim of SMOLTPRO is to develop ecologically and ethically sound methods for supplementary rearing of salmonids as well as to increase the socioeconomic returns of hatchery-produced salmon and trout. To achieve these goals, SMOLTPRO integrates the expertise and resources in this field of research using a multidisciplinary approach. Recent research have shown that modified rearing methods, such as reduced density, addition of physical structure and more nature-like feeding regimes, have positive effects on the performance of salmon and trout after release. The long-term effects of such rearing methods are, however, less well known. In SMOLTPRO, modified rearing methods will be evaluated with regard to the sea-migration of smolts, return rates of adults, and effects on the socioeconomic value. Experiments will be performed in a series of full-scale hatchery systems to evaluate the generality of effects across different climate zones. The knowledge and experience gained through SMOLTPRO will be used to develop new hatchery guidelines for sustainable smolt production, in dialogue with hatchery managers and stakeholders.

**COST 867:
International Workshop
on Fish Welfare**

**Fish welfare in
Aquaculture: The Real
World**

Chair: **Sunil Kadri**, AqualInnovation, UK

THURSDAY, FEBRUARY 10th

14:30-17:30

Session 4.



The changing agenda of animal ethics, how do fish fit in?

Peter Sandoe

Danish Centre for Bioethics and Risk Assessment, University of Copenhagen, Denmark

Traditionally the main view in the West was that animals are there for us to use and therefore there were no perceived ethical limits to animal use. This changed in the early nineteenth century when movements and legislation aimed at preventing cruelty to animals first appeared. Next, the idea of animal welfare emerged after the Second World War. The focus here changed from protecting animals from meaningless cruelty to shielding them from the adverse effects associated with intensive animal production and other forms of animal use. Lately, new developments in attitudes to animals have evolved. This have in various ways given rise to the idea that animals deserve not only protection but also respect. This development may be seen as widening rather than a wholesale change of the moral agenda governing our interaction with animals. Some animals, for example companion animals and some wild animals such as whales and elephants, according to most people in our part of the world should be treated with respect. These animals are high on what social scientists call the socio-zoological scale. Other animals, and among these are fishes, are still widely seen as mere resources for us to use. However, recently there have been some developments towards bringing fish on the moral agenda. Firstly, in aquaculture, the idea of animal welfare has gained a rather wide uptake. Secondly, there has been a growing concern for human disruption of ecosystems in the sea. In the presentation these developments will be described and it will be discussed how the moral agenda for our use of and other dealings with fish may develop in the future.

Fish welfare: a British and worldwide perspective

Michael C. Appleby

Farm Animal Welfare Council and World Society for the Protection of Animals, London, UK

The British aquaculture industry has taken a generally positive attitude to animal welfare in recent years and there has been progress on many important issues, but there are still many outstanding welfare problems. There is also overlap between these and environmental problems. These are even more clear on a worldwide basis, especially considering the sheer volume of fish catches (including for feeding farmed fish) and production. Discussion will include: differences and parallels between fish and other farmed species; differences between fish species; processes and systems; overlaps between welfare, production and food safety; and general attitudes to fish welfare.

Mediterranean farmed fish also have rights

Javier Ojeda

Asociación Empresarial de Productores de Cultivos Marinos, APROMAR, Ctra. Marquesado km 3,400; 11130 Chiclana; Cádiz; España
info@apromar.es

Aquaculture is a tough business. EU aquaculture companies develop their activities in a complex market and regulatory environment in which competition is extremely harsh. Since the year 2000 EU aquaculture has stagnated while in the rest of the world its growth has maintained an impressive pace. The reasons for this bipolar situation are complex, but can be summed up to two: the complexity for accessing new sites for farms in the EU and the inexistence of a level playing field with respect to third countries. This unlevel playing field appears in facts such as European aquaculture producers being obliged to produce their fish under stringent and expensive conditions that third country producers can fully ignore and still sell their products in the European Market. These uneven conditions comprise environmental respect, feed ingredients, workers conditions and, of course, fish welfare. Yes, Mediterranean aquaculture fish have rights. However, approving rigorous production and harvesting procedures will not assure that EU sea bream and sea bass improve their welfare. A bolder and more realistic approach is required including better availability of veterinary medicines, the accessibility to suitable sites for farm location, improved working conditions in the sea, availability of new raw materials for feeds and fair competition in the value chain.

Freedom food and aquaculture: 10 years on

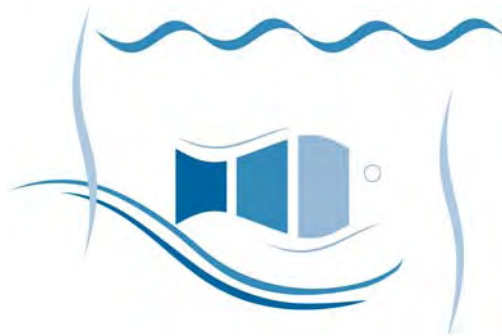
Bob Waller¹, John Aviezhinius²

¹ Agricultural Manager Freedom Food Ltd., ² Royal Society for the Prevention of Cruelty to Animals RSPCA.

Bob Waller and John Avizienius will give a presentation that will explain how the Freedom Food scheme operates they will cover the different roles that the RSPCA and Freedom Food have in developing and operating the RSPCA scheme. They will explain how they work with the industry and where the industry has benefited from the standards and what the plans are for the future. They will cover how the scheme is promoted to the consumer through raising awareness of unacceptable animal welfare practices and how they can offer an informed choice to the consumer at the point of purchase.

**COST 867:
International Workshop
on Fish Welfare**

Speaker biosketches



Daniel M. Weary

Daniel M. Weary is a Professor and NSERC Industrial Research Chair at The University of British Columbia. Dan co-founded UBC's Animal Welfare Program and co-directs this active research group. Dan's research focuses on developing behavioural measures for the objective assessment of animal welfare and developing practical methods of improving the welfare of farm animals, lab animals and wildlife.



Victoria Braithwaite

In 1992, Victoria Braithwaite graduated with a PhD in animal behaviour from the University of Oxford, UK. She moved first to a post-doc position with Prof. Felicity Huntingford at the University of Glasgow followed by a faculty position at the University of Edinburgh. In Edinburgh she developed a research program investigating fish cognition. Part of this work led to a study on pain perception and the potential for suffering in fish. In 2007 she moved to Penn State University, USA to become Professor of Fisheries and Biology. Between 2008-2011 she has been a visiting professor at Bergen University, Norway.



Neil Herbert

Dr. Neill Herbert obtained his PhD from the University of Auckland in 2002 after which he relocated to Copenhagen, Denmark, where he was part of an EU project investigating the effect of low oxygen areas on important finfish species. Whilst traditionally working within the fields of environmental physiology and behaviour, Neill subsequently expanded his work into applied areas by developing a behavioural technology aimed at improving the productivity, quality and welfare of farmed fish through sustained exercise. As a result of the research with Scottish Enterprise, the University of Glasgow and the Scottish aquaculture industry, Neill played a pivotal role in the founding of OptoSwim Technologies Ltd. With a passion for research, he is now employed by the University of Auckland and is looking forward to developing more innovative technologies for the seafood industry.



Catarina Martins

Catarina Martins is a post-doc at the University of Algarve (Portugal) and Wageningen University (The Netherlands) where she investigates the role of coping styles and dietary manipulations on the adaptive capacity of Nile tilapia and Senegalese sole. She graduated from Wageningen University with a PhD in animal sciences addressing the role of individual differences in behaviour and stress physiology in growth and feed efficiency variability in African catfish. After her PhD, Dr. Martins enrolled in a Post-Doc dealing with welfare problems in recirculating aquaculture systems, (RAS) particularly growth retardation. Her current research projects deal with accumulation of steroids in the water of RAS and the behavioural, physiological, neuro-endocrine and emotional characterization of coping styles in fish. Her primary research interests concern the study of coping styles and how these relate to cognitive appraisal and emotions in farmed fish. Dr. Martins has also a special interest on teaching presentation skills and has been providing training on this topic in several workshops for the past 3 years.



Ryan Wilkinson

Ryan Wilkinson is a Lecturer at the University of Tasmania, Australia. His current research interests involve various aspects of the endocrinology and physiology of aquatic animals (primarily fish). Recent and ongoing research projects are associated with furthering our understanding of the potentially detrimental consequences of husbandry and environmental related stressors in commercial aquaculture operations. In addition, he is interested in the hormonal mechanisms involved in seasonal reproduction in fish, photoperiod manipulation for improved aquaculture production, impacts of harvest techniques on post-harvest flesh quality and fish welfare in aquaculture. Ryan has conducted research with the Atlantic salmon, rainbow trout, barramundi, yellowtail kingfish and southern blue-fin tuna aquaculture industries, in addition to commercial feed manufacturers in Australia. His current undergraduate teaching includes the following units: Introduction to Aquaculture, Aquaculture Production, Aquaculture Hatchery Production, Aquaculture Technology, Aquatic Animal Physiology and Behaviour, Introduction to Seafood Quality and Safety, Seafood Handling and Processing.



Danielle Champagne

Danielle Champagne is a postdoctoral fellow, Department of Integrative Zoology, Institute of Biology of Leiden, University of Leiden, The Netherlands. She was educated at Laval University (Quebec City, Canada) (MA in philosophy 1996) and the University of McGill (Montreal, Canada) (D.Phil. in philosophy 2003). Since 2007, she has been developing and integrating a new line of research on zebrafish models of stress at the Institute of Biology in Leiden. Previously, she received extensive training with internationally renowned experts in the field of stress and acquired expertise in models of early-life stress (2003-2004: Prof. Michael Meaney, McGill Univ., Canada) and neuroendocrinology of the stress-regulating systems (2004-2007: Prof. Ronald de Kloet, Leiden Univ. NL). Her research interest is to understand when, where, and how stress hormones such as glucocorticoids operate during brain development to produce differential stress/adaptive and neuroplasticity phenotypes in later life. Her research program involves the use of zebrafish as both developmental model as well as behavioral model to uncover the molecular mechanisms underlying the effects of early-life adversity on the developing stress system relevant to all vertebrates, including humans (<http://www.science.leidenuniv.nl/index.php/ibl/champagne>)



John Purser

Associate Professor John Purser is currently Director of the National Centre for Marine Conservation and Resource Sustainability, Australian Maritime College, University of Tasmania, Tasmania, Australia. He has been involved in the aquaculture industry since 1985 initially managing salmon farms before moving into research and teaching at the University of Tasmania for the last 22 years. While his main focus has been on salmonids including Atlantic salmon, rainbow trout and brook charr, he has researched the production biology of other species such as greenback flounder, barramundi, galaxiids and seahorses. For a number of years his main research fields have been fish behaviour and feeding strategies and more recently how these relate to fish welfare. John also has been keenly involved in research higher degree student training having supervised around 60 PhD, Masters and Honours research students. He enjoys teaching undergraduate students about fish production techniques, live feed production, aquaculture technology and policy issues, and co-ordinates the National Centre's work placement program. John has been involved in industry training programs with Seafood Training Tasmania, sits on the Tasmanian Aquaculture Research Advisory Group and Fishing Research and Development Corporation Research Advisory Board and is a member of the University of Tasmania Animal Ethics Committee.



Anders Kiessling

Prof. Kiessling has worked with aquaculture and fish physiology since early 1980. In 1990 he received his doctoral exam, which focused different aspects of nutrition/growth physiology in muscle of farmed rainbow trout. This work was conducted in close collaboration with Akvaforsk, Norway, a collaboration link he has kept ever since. His post doc was conducted in Western Canada with DFO, West Vancouver lab. focusing different aspects of physical exercise and later adaptation of DA cannulation techniques to practical experiments. Returning to Sweden his interest changed to product quality and ethical production. Fifteen years ago (1998) he moved his professional career to Norway and worked with the Institute of Marine Research for six years before moving to the Norwegian University of Life Sciences, including also new feed sources in his research interest. From the end of 2011 he will move back to Sweden and become the Professor in Aquaculture of the Swedish University of Agricultural Sciences, focusing mainly sustainability issues of aquaculture in the Baltic Watershed.



Cedric Mathieu

In 2006 Cédric Mathieu obtained a master 1 degree in Biology from Namur University, Belgium. He achieved a thesis to study the effect of confinement stress on the immunity of Eurasian perch (*Perca fluviatilis*, L.). The year after, he successfully obtained an MSc in molecular and cellular biology and biochemistry from the University of Namur. During his MSc he achieved a training of four months as a researcher in the lab of TiGenix, a spin off from the University of Ghent and Leuven, Belgium. Since 2008, he is performing a PhD in the field of fish endocrinology in the University of Namur (Laboratory of Pr Patrick



Kestemont). The aim of his PhD thesis is to study the interaction between two corticosteroids, their receptors and the immune response of a non model species, the Eurasian perch. Few months ago, in order to characterize the perch corticosteroid receptors, he achieved a training of 6 weeks in the Institute of Aquaculture of Stirling (UK) in collaboration with Armin Sturm.

Leonor Galhardo

Post-Doc in Zebrafish Welfare (ISPA-IU, since 2011). PhD in Animal Science (fish welfare, ICBAS/ISPA-IU, 2005-2010). MSc in Applied Animal Behaviour and Animal Welfare (Univ. Edinburgh, 1993) and first degree in Biology (Univ Azores, 1990). Seven peer-reviewed papers on fish welfare published since 2005. Co-coordinator of the post-graduation course on animal behaviour and welfare (ISPA-IU, 2010-2011). Lecturing activity at ISPA-IU and at the Veterinary School of Lusofona University, Lisbon (since 2009). Animal welfare consultant for Eurogroup for Animals (Belgium, 2000-2008) and technician of the animal welfare department at the Portuguese Chief Veterinary Office (1996-1999). Coordination and lecturing in several courses on animal welfare. Development of tools to assist the implementation of the zoo directive in Portugal and in the EU. Author of several talks, technical reports and articles on animal welfare and related issues.



Rui Oliveira

Dr. Rui Oliveira (born 22.02.1966), Ph.D. 1996 (University of Lisbon), Assistant Professor in Biological Sciences 1996 (ISPA, Lisboa), Associate Professor in Biological Sciences 2001 (ISPA, Lisboa), Full Professor in Behavioural Biology 2007 (ISPA, Lisbon), Principal Investigator of the Animal Behaviour Group 2007 (Champalimaud Neuroscience Program, Instituto Gulbenkian de Ciência, Oeiras). Key qualifications in the field of Animal Behaviour and Neuroendocrinology. During the last years he has been involved in research within the field of Behavioural Neuroendocrinology and Animal Communication. Main fields of interests are (1) the social modulation of hormones, brain and behaviour in vertebrates, (2) the neuroendocrine correlates of alternative mating tactics in teleost fish, and (3) fish cognition and welfare. He leads a research group of 4 post-docs, 6 Ph.D. students, 2 lab technicians and variable numbers of M.Sc. and undergraduate students. He has experience in teaching (Biological Psychology and Neuroscience and Behaviour courses to Psychology undergraduate and Master students and advanced neuroscience and behaviour topics for the Champalimaud Neuroscience Ph.D. Programme at IGC), supervising, refereeing and evaluation of scientific research (e.g. member of the EU experts panel for the evaluation of research proposals under the Framework Programmes, reviewer for NSF Programs in Neuroendocrinology and in Animal Behavior). He is the Director of the Post-Graduate Program in Psychobiology at ISPA and PI of the Animal Behaviour Group at Instituto Gulbenkian de Ciência (IGC). He is the coordinator of four ongoing research grants at the national level and participates in one international research network. He also serves currently as Dean of ISPA, President of the Portuguese Ethological Society (SPE), Associate Editor of the journal *Hormones and Behavior* (Elsevier) and Chief-Editor of the journal *Acta Ethologica* (Springer-Verlag). He has published over 130 original papers in international peer-reviewed journals (see below for a list of selected publications) and edited 2 books.



Xavier Manteca

Xavier Manteca is associate professor of animal behaviour and animal welfare at the Department of Animal Science-School of Veterinary Science, *Universitat Autònoma de Barcelona*. He obtained his MSc degree in Applied Animal Behaviour and Animal Welfare at the University of Edinburgh and his BVSc and PhD degrees at the *Universitat Autònoma de Barcelona*. Xavier was leader of the Sub-project 3 ("Practical strategies to improve animal welfare") of the Welfare Quality Project and has been a member of several working groups on animal welfare of the European Food Safety Authority. His main areas of research are social and feeding behaviour of domestic animals, and animal welfare assessment.



Tom Pinkiewicz

Tom Pinkiewicz has received Bachelor of Computing with First Class Honours from University of Tasmania in 1999 and is currently a part-time PhD candidate at University of Tasmania as part of a collaborative research between the National Centre for Marine Conservation and Resource Sustainability, and the School of Computing and Information Systems. His main research area is aquaculture technology and his project investigates an automated analysis of fish behaviour using computer science techniques. The research leverages video technology to develop a continuous observation system in tanks and sea cages. The technology under development may assist researchers and farmers in real-time analysis of fish behaviour, alert about abnormal events and predators, and provide a tool to evaluate fish welfare. In his full-time employment, he is a software engineer with an Australian based electrical engineering company and he has been involved in the automation of a control system for aquaculture feed manufacturer Skretting Australia.



Pierluigi Carbonara

Pierluigi Carbonara was born in 1968 in Italy. He graduated at the University of Bari with a thesis on the production of phyto and zooplankton in aquaculture and obtained his PhD at the University of Siena with a thesis on fish welfare. Since 1997 he has a permanent position at COISPA. Over the past 10 years he has been mainly involved in aquaculture and fisheries biology studies, investigating in particular in the following fields: reproduction, larval and post-larval rearing of new species for aquaculture (*E. marginatus*, *P. erythrinus*, *P. pagrus*); fish welfare and swimming physiology; fecundity, maturity, reproduction and growth of the most important fisheries species in the Mediterranean. In the last five years he has been involved in studies of the swimming physiology of *D. labrax*, particularly as regards the possibility of validating muscle activity, measured through telemetric sensors, as an indicator of well-being in aquaculture.



Peter Sandøe

Peter Sandøe is professor of Bioethics, Faculty of Life Sciences, University of Copenhagen, Denmark. He was educated at the University of Copenhagen (MA in philosophy 1984) and the University of Oxford (D.Phil. in philosophy 1988). He is the director of the Danish Centre for Bioethics and Risk Assessment (CeBRA), an interdisciplinary and inter-institutional research centre founded in January 2000. Since 1992 he has served as Chairman of the Danish Ethical Council for Animals, an advisory board set up by the Danish Minister of Justice. From 2000 to 2007 he was president of The European Society for Agricultural and Food Ethics. From 2009 he has been Special Professor of Animal Ethics at the University of Nottingham. Since 1990 most of his research has been in bioethics, with particular emphasis on ethical issues relating to animals, biotechnology and food production. He is committed to interdisciplinary work combining the perspectives of natural science, the social sciences and philosophy. He has published 74 papers in journals with peer review; he has made in excess of 200 other academic contributions and more than 150 contributions aimed at a broader audience.



Micheal Appleby

Michael Appleby took a BSc in Zoology at the University of Bristol and a PhD in Animal Behaviour at the University of Cambridge. He then carried out research at the Poultry Research Centre in Scotland and the University of Edinburgh for 20 years on behaviour, husbandry and welfare of farm animals. Publications include six books as author or editor, among them *Animal Welfare* (1997) and *Long Distance Transport and Welfare of Farm Animals* (2008), and Michael has lectured in more than 25 countries worldwide. From 2001 to 2005 he was head of the Farm Animals and Sustainable Agriculture section of The Humane Society of the United States in Washington, DC. He returned to the UK to work on Trade Policy with the World Society for the Protection of Animals and other NGOs. He now works as Chief Scientific Adviser for WSPA. Dr Appleby is a member of the Farm Animal Welfare Council and a Visiting Professor at the University of Plymouth and the Scottish Agricultural College.



Javier Ojeda

Javier Ojeda holds a BSc in General Biology obtained at the Universidad Autónoma of Madrid, Spain (1986), and a Master of Science in Marine Sciences from the University of South Carolina, USA (1989). Since 1989 he has been involved in production in aquaculture. He has worked in several fish farms in Spain and Ireland, both in hatcheries and ongrowing farms, mainly for sea bream, sea bass and salmon. He has also been available as aquaculture consultant. In 2003 he was appointed general manager for APROMAR, Spain's marine aquaculture farmers association. From this position he serves not only at a national level but also participates in international forums such as the European Commission, the European Parliament and the European Social and Economic Committee. He has also been involved in several projects for FAO and other organisations mainly on environmental, sustainability and certification issues. Presently Mr Ojeda is vice chairperson of Working Group 2 of the Advisory Committee for Fisheries and Aquaculture of the European Commission.



Bob Waller

Bob trained in agriculture at Chadacre Agricultural Institute in Suffolk and then spent 24 years in farm management cover all aspects of agriculture on several farms throughout the UK. He joined Freedom Food in 1999 and became technical development manager in 2000 and moved to agricultural manager in 2010. Bobs roll within Freedom Food is to offer technical support on all the species within the scheme to all areas of the business to the assessors on the implementation n of the RSPCA Freedom Food standards to the marketing teams promoting Freedom Food to the retailers. He is also first to meet groups and individuals wishing to understand how the Scheme operates and how the standards are implemented on farms and what the benefits are to the producers. Bob worked closely with the RSPCA in the development of the RSPCA standards and works closely with the RSPCA on the development of the standards into other areas of the Scheme.



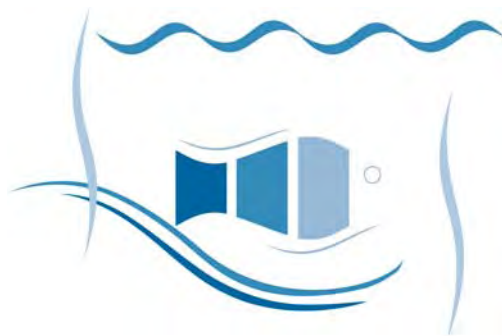
John Avizienius

I was a stockman on terrestrial faros for 10 years, followed by 3 years as a farm management consultant. Presently, I am Deputy Head of Farm Animals at the Royal Society for the Prevention of Cruelty to Animals (RSPCA), with lead responsibility for aquaculture and cattle, which includes writing the RSPCA standards for farmed salmon which are then used by the Freedom Food Scheme. I am also a member of the European Animal Welfare Platform fish cluster.



**COST 867:
International Workshop
on Fish Welfare**

Poster sessions



Melatonin reduces cortisol release in cultured head kidney of goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*)

Clara Azpeleta¹, Gert Flik², Fernando Torrent³, Nuria de Pedro¹, Elena Velarde¹, Aída Sánchez-Bretaño¹, María Jesús Delgado¹

¹Department of Physiology (Animal Physiology II), Faculty of Biology, Complutense University, Madrid, Spain; ² Department of Organismal Animal Physiology, Faculty of Science, Radboud University, Nijmegen, The Netherlands; ³ Department of Forest Engineering, ETSIM, Polytechnic University, Madrid, Spain. E-mail: mjdelgad@bio.ucm.es

Previous results in goldfish (*Carassius auratus*) have reported that melatonin (5-methoxy-N-acetyltryptamine, MEL) intraperitoneally administered counteracts the increase in plasma cortisol levels induced by exposure to acute stress. Our objective was to determine whether MEL-induced reduction in plasma cortisol levels is mediated via a direct action of this indoleamine on interrenal steroidogenic cells in fish. With this aim we used two different *in vitro* approaches on two teleost species, a superfusion system for the culture of the goldfish head kidney, and a static system to rainbow trout (*Oncorhynchus mykiss*) head kidney cultures. Tissues were incubated in the presence of adrenocorticotrophic hormone (ACTH, 50 nM for superfusion, 1.3 µM for static), ACTH plus MEL (dose response) and ACTH+MEL+luzindole (a general MEL receptor antagonist, 1 µM). Medium was withdrawn at different time points (from 0 to 8 hours) and preserved for posterior cortisol measurement. MEL addition (100 nM) to the culture medium reduced the height of the maximum peak of cortisol release in ACTH-stimulated goldfish head kidney. Similar results were obtained in trout head kidney, with a reduction in ACTH-induced total cortisol release by MEL (10 nM), particularly at 4 hours after hormones addition. The luzindole blocked such effect of MEL. Present results show a counteracting role of MEL on head kidney activation by the ACTH, supporting previous results of cortisol reductions by MEL on *in vivo* experiments in goldfish. This is the first report in fish demonstrating a direct and specific action of this indoleamine on interrenal steroidogenic cells.

Conditioned food anticipatory behaviour as indicator for stress and welfare in farmed atlantic salmon

Ole Folkedal*, Thomas Torgersen, Frode Oppedal, Lars H. Stien, Rolf Erik Olsen, Anders Fernö, Anne Aasjord, Jonatan Nilsson, Victoria A. Braithwaite, Tore S. Kristiansen.

Institute of Marine Research. NO-5984 Matredal, Norway.

ole.folkedal@imr.no

Given the growing consensus and current legislation on fish welfare, fish should be regarded as sentient beings and be treated accordingly. Welfare should therefore ideally be assessed by their sentient attributes of emotional states. Methods for such assessment in aquaculture are, however, not available. We have investigated one possible way of indirectly measuring fish's experience of life, by tapping into their motivational systems and look at their sensitivity for reward. The behavioural choices an animal makes are considered to be controlled by hedonic mechanisms set to maximize reward on the basis of its internal and external environment. This requires a continuous subjective evaluation to select the appropriate (most rewarding) behaviour at any time and in any situation, which is determined by the state of an animal's motivational systems (e.g. hunger, thirst, avoidance, fear). The motivational state of an animal can indirectly be assessed by measuring anticipatory behaviour when it is expecting a positive stimulus (e.g. food) such as in a Pavlovian conditioning regime (Spruijt et al., 2001). The present work hypothesized that groups of salmon, conditioned to respond to a light cue signalling food arrival, would show less anticipatory behaviour (fish crowding in the feeding area) when in a state of stress, i.e. that stress coping will be higher prioritized. Accordingly, the strength of conditioned anticipatory behaviour may be used as a non-invasive tool to assess how strong and for how long an aversive experience influences the behaviour.

The results from a series of tank experiments revealed reduction in conditioned anticipatory behaviour as a sensitive indicator of stress level. Groups of salmon reared at commercial densities at both the parr and post-smolt life stages showed reduced anticipatory behaviour after being exposed to a range of relevant acute stressors. Recovery was found slower than that of physiological parameters of elevated cortisol release to water and altered oxygen consumption. The anticipator behaviour was noticeably different at the life stages, probably reflecting their natural feeding strategies. Additionally, the method was found useful to assess the partial adaptation of post-smolts to an aversively high temperature given in a fluctuating regime over weeks. Weak anticipatory behaviour was then quite surprisingly accompanied by feeding at near control level. This indicates that the method is sensitive towards measuring a persistent challenging state, and that it is a more sensitive indicator of stress than feed intake which is a commonly used welfare indicator in aquaculture.

Melatonin, vasotocin and isotocin as biomarkers of the condition of fish

E. Kulczykowska¹, M. Gozdowska², J.A. Martos-Sitcha³, H. Kalamarz⁴, M. Nietrzeba⁴, J.M. Mancera⁵ and G. Martínez-Rodríguez⁵

¹*Pomeranian University, Poland, Institute of Oceanology PAS, Sopot, Poland* ²*Institute of Oceanology PAS, Poland,* ³*Universidad de Cádiz, Instituto de Ciencias Marinas de Andalucía, CSIC, Puerto Real, Spain,* ⁴*Institute of Oceanology PAS, Poland,* ⁵*Universidad de Cádiz, Puerto Real, Spain,* ⁵*Instituto de Ciencias Marinas de Andalucía, CSIC, Puerto Real, Spain*
ekulczykowska@iopan.gda.pl

The environmental changes interfere with vertebrate endocrine systems. The endocrine biomarkers are used to indicate the condition of both free-ranging and farmed animals. The aim of this study was to show an impact of environmental changes on arginine vasotocin (AVT), isotocin (IT) and melatonin (MEL) in selected fish species. AVT and IT are neurohormones synthesized in hypothalamic nuclei and released to the blood stream in the neurohypophysis. Changes in hypothalamic, pituitary and plasma AVT and IT concentrations were found in many fish species subjected to different type of stress, i.e. confinement, disturbance, high density, food deprivation or osmotic. An effect of stress on nonapeptides' synthesis and release depends on the type of stress stimuli. MEL, synthesised mainly in pineal organ, is a well-known scavenger of free radicals and serves as a sensitive, first-line defensive molecule in the protective mechanism of organisms. Thus alterations in pineal and plasma MEL levels could be useful biochemical markers of exposure to stress. A role of AVT, IT and MEL in stress axis of fish has been suggested. AVT, IT and MEL can be considered as hormonal markers of the internal state of the individual and thus seems to be good candidates for welfare indicators.

Hypoxia tolerance and habituation of atlantic salmon (*Salmo salar* L.) subjected to fluctuating hypoxia

Mette Remen, Rolf Erik Olsen, Thomas Torgersen, Turid Synnøve Aas, Tone Vågseth, Frode Oppedal
Institute of Marine Research, Matre, 5984 Matredal, Norway.
Mette.Remen@imr.no

Recent environmental studies of Atlantic salmon sea cages show that hypoxia occurs and fluctuates at both short- and long-term scale, primarily in the autumn period. It is expected that such periods constitute a problem for fish welfare and production efficiency, but literature is scarce on hypoxia tolerance of Atlantic salmon. The ability of Atlantic salmon to cope with and habituate to hypoxic periods was investigated through two experimental trials. Oxygen levels fluctuated between normoxia (90% DO) and hypoxia (70, 60, 50 and 40% DO) four times a day at 16°C. Short-term responses (stress physiology, appetite, metabolism and respiration) and long-term responses (changes in metabolism, feed utilization and growth) were measured and critical oxygen levels established. Initially, hypoxia induced a stress response in the 40 and 50% DO groups together with reduced feed intake. Feed intake was closely related to hypoxic level throughout the experiment, resulting in reduced growth and condition factor. Habituation was indicated by a down-regulated stress response and an increased feed intake in normoxic periods. During long-term exposure (10 weeks) to hypoxic periods, the effect of hypoxic periods on appetite and growth persisted, but no clear effect was seen on feed utilization. Data on changes in metabolic rate and critical oxygen levels will be presented.

Effects of chronic confinement stress on growth, survival, blood cortisol and glucose of perch (*Perca fluviatilis*)

C.Rougeot¹, T.Tomson, M. Vandecan¹, S.Milla², S.M.N Mandiki², P. Kestemont² and C. Mélard¹
¹*Aquaculture Research and Education Center (CEFRA), The University of Liège, Chemin de la Justice 10, B-4500 Tihange, Belgium.* ²URBO, FUNDP, 5000 Namur, Belgium
C.Rougeot@ulg.ac.be

In order to study the effect of chronic confinement encountered in intensive rearing system (RAS) on juvenile (MBW : ± 35 g) Eurasian perch (*Perca fluviatilis*), three levels of increasing confinement with the same density (500 fish/m³) were used in the experimental setup: two 1600L tanks (low confinement, surface of 4m²), two 800L tanks (medium confinement, surface of 2m²) and two 144L tanks (high confinement, surface of 0.36m²) at $22.8 \pm 0.4^\circ\text{C}$, and $\text{O}_2 > 7.0$ ppm. Fish were fed with an automatic feeder, the food ratio was adapted each day. Fish were controlled (total biomass) every 3 weeks, and experience lasted for 140 days. Blood indicators of stress (plasma cortisol and glucose) were measured two times at the end of the experiment (J110 and J139) by collection of blood in the caudal vein after anesthesia with phenoxyethanol (0.4ml/l) and within 5 minutes after capture. Growth was significantly decreased with the increase of confinement (final mean body weight of 93g and 74g for 1600L and 144L respectively) as well as the survival rate (96% and 85% for 1600L and 144L respectively). Glucose and cortisol levels were decreased with the increase of confinement. In conclusion chronic confinement induced a change in plasma cortisol and glucose level and significantly reduced survival and growth in Eurasian perch.

Effects of chemical and handling stress on oxidative stress in gilthead (*Sparus aurata*)

MJ Sánchez-Muros, S. Villareces, C de Haro, F Garcia-Barroso
Department of Applied Biology . University of Almería. Spain
mjmmuros@ual.es

The formation of free radicals is related with the detoxification process, in fish the oxidative stress is a useful index of stress for contamination of xenobiotic compound. It is described that the response to oxidative stress and the antioxidant potential are different for different habitat or feeding behaviour. This capacity to adapt the response to different stimuli could be useful to discriminate the different types of stress. This work studies the ROS and FRAP production as oxidative stress index under two stress conditions, a soft weekly exposure to pesticide and handling stress in order to identify some parameters to help know the welfare status. 75 immature of *Sparus aurata* were distributed in 3 different tanks (25fish/tank).; control, chemical stress and handling stress The chemical stress was induced by 24 hour exposition each week to a 0,20mg/l of a pesticide. The handling stress was caused by putting a net into the tank once a day for 30 sec., at different times every day. The experiments lasted for 15 days Oxidative stress was evaluated as ROS and FRAP production in muscle, liver, digestive and gill The two stress situations assayed provoked changes in FRAP levels in all organs studies. Regarding the ROS level no statistical differences were found for digestive and gill In muscle only Diuron shows a significant increase of ROS whilst in liver ROS levels increase two stressing groups. Diuron affects especially the antioxidant response of the gill. Handling stress, all the organs lost the capacity of a proportional response.

Evidence for chemical communication in Nile tilapia: disruption of feeding behaviour by alarm cues

Patrícia I.M. Silva*, Catarina I.M. Martins, Erik Höglund and Øyvind Øverli

¹ *Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Aas, Norway*, ²Technical University of Denmark, National Institute of Aquatic Resources, North Center, DK-9850 Hirtshals, Denmark, ³ Centro de Ciências do Mar (CCMar), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
patricia.silva@umb.no

The Nile tilapia is a freshwater cichlid, which is produced worldwide yielding approximately 2.5 million tons year (including all tilapia species). Several recent publications have shown that consistent individual variation in correlated traits, so called behavioural syndromes or stress coping styles, are present in fish and can have large consequences for fish welfare and performance in aquaculture. Furthermore, fish exhibit threat-sensitive behaviour responses aiming to avoid predation and dominant fish by moving away from the alarm cue, and decrease movement (freezing or hiding). The purpose of this study was two-fold: To identify behavioural traits which are consistent over time in individual tilapia, and investigate whether such traits predict the behavioural response to acute stress or to conspecific alarm cues. Latency to take distributed food was found to be one of such traits, which was correlated to routine locomotor behaviour but not to the locomotor response to acute stress. Feeding latency also predicted bottom-grazing behaviour, a trait that the introduction of conspecific alarm cues inhibited without disrupting the behavioural correlation.

Apparent digestibility as a potential indicator of welfare in farmed fish

Cotou E. and Nengas I.

Institute of Aquaculture, Hellenic Centre for Marine Research (HCMR), Agios Kosmas Hellinikon, 16604, Greece.

ecotou@ath.hcmr.gr, jnegas@ath.hcmr.gr

The physiological, health and/or behavioral status of individual fish have been used by some scientists as indicators of compromised welfare, though the link between components of the stress response and welfare is not simple. Stress responses are useful as indicators of the impairment of the normal welfare conditions because they represent a natural reaction to adverse environmental conditions. It has become apparent that replacement of fish meal by cheaper ingredients of either animal or vegetable origin in feedstuffs is necessary due to the rising cost and uncertain availability of fish meal. Feed composition is important for preserving fish welfare since inappropriate composition of food could cause the impaired of fish welfare. Feeds contain nutrients and energy sources essential for fish growth, reproduction, and health. Research has shown that the quality of different feedstuffs is greatly dependant on nutrients and their digestibility. However, not all nutrients are digestible for fish. The term 'digestibility' includes three elements; completeness of solution & absorption of the food nutrients, rate of digestion, and comfort of digestion. Digestibility is one of the most important aspects in evaluating the efficiency of feedstuffs. Determining the digestibility of nutrients is important not only to enable formulation of diets that maximize the fish growth by providing appropriate amounts of available nutrients but also to limit the wastes produced by the fish. In some instances the assessment of digestibility can identify problems where further assessment strategies applied have shown why such problems are occurred (distal enteritis). Based on results from the international literature we investigated the application of "apparent digestibility" as a potential indirect indicator of health and welfare of farmed fish.

Welfare of farmed rainbow trout *Oncorhynchus mykiss*, preferences for stocking density

Danielle Caroline Laursen*, Madelene Åberg-Andersson and Erik Höglund

Danish Technical University, Department of Aquatic Resources, Section for Aquaculture, North Sea Center, P.O.Box 101, DK-9850, Hirtshals, Denmark

*dcla@aqua.dtu.dk

In the aquaculture industry, fish are exposed to a range of unfavourable environmental conditions. Amongst these, high stocking densities have attracted a lot of attention due to the increasing concern for the wellbeing of fish in aquaculture. Limited scientific evidence has suggested that stocking densities may compromise welfare and ultimately impact production. The recommendations for stocking rainbow trout (*Oncorhynchus mykiss*) in production systems are somewhat elusive, ranging from 2 to 80 kg/m³. Furthermore, this advice is usually based on intuition and previous experience. Therefore, the aim of this study was to investigate the preferences of rainbow trout for stocking density. This was achieved using two-choice systems, each system consisting of two tanks attached via a doorway. The distribution observed in this system was one tank occupied by a few dominant individuals and the majority of the fish preferring to occupy the second tank. This choice between being exposed to aggression by dominant individuals versus crowding was utilized to study preferences for rearing densities. Fish were stocked at 20, 40 and 80 kg/m³. The doorway was left open and the fish moved freely between the two tanks for three days, after which the number of fish in each tank was determined. There was a clear relationship between the frequency of fish entering the "dominant tank" and rearing density. In fish showing a preference for the "crowded tank", there was a positive relationship between density and plasma cortisol. Taken together, this demonstrates that crowding compromises welfare in rainbow trout.

A potential non-invasive indicator of stress levels in aquarium fish

A Spiess*§; T Carter*; DM Broom§; T Ellis‡ & AD Scott‡

* [Department of Life Sciences, Anglia Ruskin University, East Road, Cambridge, CB1 1PT](#), § Centre for Animal Welfare and Anthrozoology, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 0ES and ‡ Cefas, Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB

toby.carter@anglia.ac.uk

This study examined the potential of using electric organ discharges (EODs) as a noninvasive indicator of stress in black ghost knife fish (*Apteronotus albifrons*, Apterontidae, Linnaeus). Characteristics of the EODs, EOD amplitude and EOD frequency, were recorded alongside water cortisol concentrations before and after exposure to a potential stressor (air exposure). Fish from both the test group (air exposure) and a control group (no air exposure) increased their EOD frequency after repeated transfer between tanks, with and without air exposure. Only the test group significantly increased their EOD amplitude following transfer between tanks. The significant increase in EOD amplitude shown by the test group was accompanied by a greater increase in water cortisol concentration, compared with the control group. This study provides the first indications that EOD amplitude increases after a stressful event to a higher degree than EOD frequency and that it could be a reliable non-invasive indicator of stress in black ghost knife fish.

Aquaculture: the fish's viewpoint

Paul Denekamp

[Board member of de Stichting Vissenbescherming \(the Foundation for the Protection of Fish\)](#)

[Nieuwe Westerdokstraat 62 1013 AG Amsterdam.](#)

pauldenekamp@hotmail.com

As Foundation for the Protection of Fish, we consider what happens to fish in the seas but also in the aquaculture as the biggest infringement of welfare of all vertebrates. Because of this infringement aquaculture is often compared with factory farming. Much needs to be changed in aquaculture in terms of the treatment of fish to make it acceptable and for this change science can be very useful. For animals that are kept it's very important for their welfare that they are able to develop their natural behaviours. In factory farming that's mostly impossible for them. What the natural behaviour is of the numerous fish species in aquaculture is often even not known. Until now science didn't show enough ambition to investigate this natural behaviour of fish profoundly. If we operate on the basis of the 'precautionary principle', no fish species should be kept before it is made clear what captivity means for him and what natural behaviours it would normally perform. We like to prevent experiments on how to keep various fish species when, from the standpoint of animal welfare, these experiments should never take place. And we want scientists to develop indicators that give animal protectionists clear perspectives on the welfare of the captive fish. In aquaculture, standards for quality fish will play a bigger role. Our fight is that welfare issues and the possibility of developing natural behaviour must be included in these standards. Science can help a lot by developing good criteria to measure welfare.

Enzymatic changes in the wedge sole (*Dicologlossa cuneata*) due to crowding stress

Marcelino Herrera¹, Luis Vargas-Chacoff^{2,3}, Ismael Hachero¹, María L. Cordero¹, Ignacio Ruíz-Jarabo², Juan M. Mancera²

¹ IFAPA Agua del Pino. Ctra. Cartaya-Punta Umbría. 21459 Cartaya (Spain). ² Departamento de Biología. Universidad de Cádiz. 11510 Puerto Real (Spain). ³ Instituto de Zoología. Universidad Austral de Chile. Valdivia (Chile).

In previous short-term experiments, it has been demonstrated that wedge sole shows some physiological responses (hyper- or hypoglycemia, liver glycogen depletion, etc.) to stressors as extreme salinities and high stocking densities, though neither long-term experience has been made no enzyme activities have been assessed in these type of works. Therefore the objective of this work was to assess the physiological changes due to stocking density, focusing on biometry, plasma metabolites and tissular enzyme activities. Wedge soles (0.21 ± 0.02 g; 5.76 ± 0.21 cm) were cultured in 125 L PRFV tanks for 197 days. The experimental stocking densities were 250, 500 and 1000 fish m^{-2} (LSD, MSD, HSD respectively). Survival and growth were periodically registered, and plasma, muscle and liver samples were extracted at the end of the experience. Survival was higher in LSD though growth was not statistically different among treatments. Plasma metabolites did not significantly vary with stocking density. The long length of the culture could induce a situation of chronic stress that could explain the absence of differences in plasma parameters. However, liver hexokinase activity was higher in HSD, suggesting a glycolysis enhancement in this treatment. In muscle, enzyme activities grew in parallel with stocking density. This fact could be related to a higher swimming activity in fish submitted to crowding stress. This work has been financed by project INTERREG 0251_ECOAQUA_5_E.

Preliminary experiments in zebrafish (*Danio rerio*) and European sea bass (*Dicentrarchus labrax*) with FishRest, a new anesthetic for fish

Esmail Lutfi, Francesc Padros and Lluís Tort.

Dpt. Cell Biology, Physiology and Immunology. Universitat Autònoma de Barcelona. 08193-Bellaterra. Spain

The anaesthetic efficacy of *FishRest* was evaluated in zebrafish (*Danio rerio*) and European sea bass (*Dicentrarchus labrax*). *FishRest* is an herbal anaesthetic formulation containing eugenol and other compounds. The preliminary trials with this anaesthetic formula showed a correct effectiveness and a large margin of safety which also indicates a similar effect than another natural anesthetic, the clove oil. In the experimental set up, the time for acquisition of anaesthesia (A3 and A5) and the recovery from anaesthesia (R3 and R5) were recorded for both species. *FishRest* at the concentration of 60 ppm showed similar results than other anaesthetics currently used, showing induction times of approximately 3 minutes, and recovery times between 6 and 8 minutes either for zebrafish or European sea bass ($T^{\circ} 25 \pm 2^{\circ}C$ y pH $7,5 \pm 0,2$) at the concentration of 40 ppm ($T^{\circ} 24 \pm 2^{\circ}C$ y pH de $7,9 \pm 1$). The anaesthetic did not show any correlation between induction time at the stage 5 of anaesthesia and weight, length or condition factor, for zebrafish. The absence of large differences in the behavioural effect of *FishRest* between zebrafish and European sea bass, allows us to suggest that its application to other species may give similar results. All together, the present study suggests that *FishRest* may become a natural and effective alternative to anaesthetics used nowadays in aquaculture,