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A.3–INTEGRATION OF BEHAVIOUR AND PHYSIOLOGY
Organised by K.A. Sloman, R.W. Wilson and K.M. Gilmour

A3.1 Hormonally-derived sex pheremones in fish: synchronising endogenous and exogenous conspecific functions

N. Stacey (Department of Biological Sciences, University of Alberta)

Vertebrate sexual behaviors typically are regulated by two general actions of reproductive hormones: actions on the brain that synchronize an individual's behavior with gamete maturation, and actions on neural and non-neural effectors that signal reproductive status to conspecifics. In addition to these endogenous hormonal actions, fish commonly employ hormones and related compounds as exogenous cues and signals (*hormonal pheromones*), a consequence of life in a medium that can limit transfer of visual information but which readily exposes the olfactory organ to hormonal compounds obligatorily released by conspecifics. Water-borne steroids, prostaglandins and their metabolites are detected with great sensitivity and specificity by the olfactory organs of diverse fishes, and exert important effects on reproductive behavior and physiology in major taxa including carps (goldfish), catfishes, salmon, and gobies. Best understood are goldfish, in which the periovulatory female sequentially releases a preovulatory steroid pheromone and a postovulatory prostaglandin pheromone that dramatically affect male behavior, physiology, and reproductive fitness. Because these hormonal pheromone systems chemically link endocrine and nervous systems among conspecifics, they challenge classical concepts that sex hormone actions are restricted to the individual. Moreover, given the chemically conserved nature of vertebrate reproductive hormones, and the diversity of fish mating and gender systems, the hormonal pheromone systems of fish not only raise fascinating questions about sex pheromone function and evolution but also provide numerous tractable models for seeking the answers.

A3.2 Integrating chemoreception, mechanoreception and odour current generation in the search for food and mates

J. Atema (Boston University Marine Program, Woods Hole)

At the small spatial scales of cell biology diffusion is fast and rapid chemical information exchange is critical to life. At intermediate scales up to centimetres, currents can still move chemical signals fast enough to guide behaviour directly: lobsters may be at the upper limit of using spatial information contained in odour plumes and using jets for communication. At this intermediate scale, chemoreception and mechanoreception are linked in interesting ways. The taste-tactile systems provide a good example of joint body/brain mapping: to find the pellets among the pebbles in the mouth. The olfaction-hydrodynamic systems may be linked as well but without the body projection. Instead, their bimodal reinforcement may keep animals on track when following interesting wakes. The temporal tuning of chemo and mechanoreceptor cells should show intermediate bandwidths corresponding to the temporal properties of biologically relevant plume structures. At even larger scales and speeds, other senses begin to play increasingly important roles and may supplant chemoreception entirely. In the fast world of light and acoustic pressure transmission we find linked brain maps of the external world. Such maps are not expected for the slow senses. Thus, for chemo/mechanosensory lobsters, the daily world may be only a few body lengths in size, sufficient to probe with antennae and gill currents. If so, one must wonder if and how they guide their migrations and local homing; perhaps a long sequence of body lengths adds up! Artificial human senses now probe beyond our world creating another map…of the Universe.

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A3.3 Dominance fights, urine communication and a possible role of biogenic amines as pheromones in crayfish

T. Breithaupt (University of Hull)

In crayfish, as well as in many other invertebrates and vertebrates, the emergence of dominance hierarchies constitutes an important social mechanism of reducing the risk of injury to individuals. We showed that urinary chemical signals play an important role for establishing dominance. Through visualisation of urine signals in fighting pairs of *Astacus leptodactylus* we demonstrated that agonistic actions are only effective in reducing the opponents' aggression when they are accompanied by urine signals. Concordant with this, we now found that competitions of *Procambarus clarkii* take much longer when the combatants are prevented from releasing urine. Second fights are generally shorter due to low resistance of the loser. This effect could result from differences in confidence by the combatants (confidence hierarchy) or from sensory cues communicated between them (assessment hierarchy). Either mechanism could be mediated by biogenic amines (e.g. serotonin, see Huber and Delago, 1998, J.Comp.Physiol. A 182: 573–583). By catheterising previously fought crayfish *P. clarkii*, we blocked the urine release in second fights and collected urine for chemical analysis. The block of urine communication prevented the maintenance of dominance indicating that dominance was mainly controlled by chemical signals (assessment hierarchy), and less so by self-reinforcing effects. Using HPLC with electrochemical detection we found that most urine samples contained serotonin-O-sulfate, a metabolite of serotonin, but we did not find octopamine or dopamine. Current investigations aim to find out whether there are differences in serotonin metabolite level between dominant and subordinate animals.

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A3.4 Evidence for functional asymmetry in the olfactory system of the Senegalese sole (*Solea senegalensis*): Behavioural implications?

Z. Velez (Centro de Ciências do Mar, Universidade do Algarve), P.C. Hubbard (Centro de Ciências do Mar, Universidade do Algarve), E.N. Barata (Centro de Ciências do Mar, Universidade do Algarve) and A.V.M. Canário (Centro de Ciências do Mar, Universidade do Algarve)

In contrast to most fish species, the two olfactory epithelia of the family Soleidae are essentially in contact with two different environments; the upper (right) side samples open water whilst the lower (left) side samples interstitial water. The present study assessed whether there are differences in the types of compounds to which the two epithelia are sensitive by use of the electro-olfactogram in the Senegalese sole (*Solea senegalensis*). The upper epithelium was significantly more sensitive to the aliphatic amino acids L-alanine, L-glycine, L-threonine and L-serine than the lower epithelium. The lower epithelium was significantly more sensitive to aromatic amino acids such as L-tryptophan, L-tyrosine and L-phenylalanine. Both epithelia had similar sensitivity to basic amino acids (L-arginine and L-lysine) and sulphur-containing amino acids L-cysteine and L-methionine. Neither side was sensitive to acidic amino acids (L-aspartate and L-glutamate) nor the D-isomers of any amino acid tested. The upper side was much more sensitive to conspecific-derived stimuli (bile and intestinal fluid) than the lower side. We suggest that these differences in sensitivity are related to different functional roles in the location (upper) and identification (lower) of potential food items; the upper olfactory epithelium is likely to be more involved with chemical communication than the lower.

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A3.5 Integrating fish behaviour, physiology and growth: the role of parasites

I. Barber (University of Wales Aberystwyth)

Understanding the impact of parasites on patterns of host behaviour, growth and physiology is facilitated by the use of experimental systems. In a series of studies, we experimentally infected three-spined sticklebacks, *Gasterosteus aculeatus*, with the cestode *Schistocephalus solidus*, which grows rapidly in the fish coelom and requires the stickleback to be eaten by a bird to complete its lifecycle. First, we examined the effect of ration on host and parasite growth. Our results suggested that whereas experimentally-infected fish grew less well, and were nutritionally more stressed, at all ration levels, the relationship between host ration and parasite growth was more complex, with parasites apparently benefiting from intermediate host rations. We then investigated whether experimentally-infected fish were able to undertake compensatory growth following a period of food deprivation. Our results suggested that infected fish could not compensate completely for early growth deficiency, with an inability to undertake the required hyperphagia being a probable mechanism. In the final set of experiments, we examined the shelter use behaviour of sticklebacks over the course of infection, linking parasite ontogeny to host behaviour. Our results suggested that behavioural change in parasitised fish was linked closely to the mass of the parasite involved, suggesting that

physiological changes in the host – correlating temporally with worm development – may be responsible for behavioural changes that may enhance parasite transmission. I conclude by discussing the various types of physiological mechanisms that may be involved, and some of the approaches we are taking to understand further this uniquely amenable system.

A3.6 Interactions between animal physiology and cognition

V. Braithwaite (School of Biological Sciences, University of Edinburgh)

Animals are constantly faced with decisions, for example, who to mate with, or, where to forage? To make adaptive decisions the animal relies on its cognitive ability. Animal cognition is generally considered to involve perception, learning and memory. Thus, when faced with a decision, we can understand the animal's responses in terms of how it uses its various sensory systems to perceive the current environment, and how it then compares its current situation with previously remembered experiences. We have been working on a number of different animal model systems to investigate how cognitive abilities are affected by the environment and by the animal's current physiological state. I will present work that highlights how hormones can influence cognitive ability in a number of ways. For example, we now have evidence that the reproductive state can influence how sensitive an animal is to certain aspects of coloration. Similarly we have investigated how sex hormones influence learning and memory processes. Together these approaches highlight how fine-tuned an animal's cognitive ability is to its current physiological state.

A3.7 Abstract not supplied

A3.8 Using physiological data to assess animal well-being

P. Hawkins (Research Animals Department, RSPCA, UK)

The requirement to minimise animal pain, suffering and distress associated with scientific procedures is widely accepted and is included in the SEB Code of Conduct. However, the techniques routinely used for recognising and assessing research animal wellbeing and adverse effects require subjective judgements. This is a concern because pain-coping behaviours in many species can be extremely subtle or not apparent at all to human observers. There is thus a pressing need for more objective indicators of discomfort and distress.

Physiological data obtained during experiments can be usefully applied to assess animal wellbeing. One example is telemetry, which can provide data on variations in heart rate, blood pressure and body temperature that may reflect acute or chronic discomfort or distress. This can be an extremely useful tool to help interpret behaviour in response to both experimental procedures and animal husbandry refinements. Telemetered variables such as body temperature can also indicate when intervention is necessary to reduce suffering following surgery, during toxicity tests or when animals are used as disease models.

It is important to consider how physiological data could be used to assess well-being wherever possible, so that animals can benefit from more effective monitoring. This will also help to improve the quality of the resulting science, as investigators using this approach can be more confident that their results are obtained from animals who are not experiencing pain or distress.

A3.9 Swimming energetics in the grey mullet: laboratory and field approaches

C. Lefrançois (IMC-International Marine Centre, Italy), D. Webber (VEMCO Ltd. Canada), R.S. Ferrari (IMC, Italy), A. Satta (IMC, Italy) and P. Domenici (CNR, Italy)

This study aimed at investigating the swimming energetics of the grey mullet (*Mugil cephalus*). To estimate the optimal and the critical swimming speeds, we employed a common swimming step-increase protocol using a swimming respirometer. In order to investigate swimming activity in the field, we tested in parallel an innovative approach based on caudal differential pressure. Fish (total length: 38.7 ± 2.4 cm; weight: 0.62 ± 0.11 kg) were tagged with pressure sensors and swam individually. A test was performed using a step-protocol involving 10 $cm.s^{-1}$ velocity increments every 30 minutes until exhaustion. At each step, oxygen consumption (MO_2) was recorded. Caudal differential pressure was continuously monitored during each experiment. Preliminary results show that the increase in swimming speed (from 0.2 to 2.7 body lengths/s) was accompanied by an exponential increase in differential pressure (from 100 to 800 pascals) and MO_2 (from $\sim$ 205 to 1015 $mgO_2.h^{-1}.kg^{-1}$). The results obtained from the laboratory experiments suggest that the caudal differential pressure can be used as an estimator of both swimming speed and the associated energetic expenditure in grey mullets. Preliminary tests in the field were carried out in September 2003 using acoustic transmitters recording caudal differential pressure. Two fish were tagged and released in a local lagoon. Based on the field data and the laboratory experiment, a holistic view of the grey mullet swimming energetics will be presented and discussed.

A3.10 Locomotory behaviour and post-exercise physiology in relation to swimming speed, gait transition, and metabolism in free-swimming smallmouth bass *Micropterus dolomieu*

S.J. Peake (Department of Biology, University of New Brunswick, Fredericton, NB, Canada) and A.P. Farrell (Department of Biology, Simon Fraser University, Burnaby, BC, Canada)

We studied behaviour, locomotory gait transition, and post-exercise physiology of smallmouth bass that voluntarily ascended a 25 m raceway against water velocities ranging from 40 to 120 cm/s. Our first objective was to link exercise intensity (i.e. mean swimming speed) to metabolism using behavioural indices (ground speed, ascent rate, success rate, and time between consecutive ascents), gait transition dynamics, and established relationships between gait use and muscle recruitment and energetics. Our second objective was to use post-exercise physiological measurements (muscle glycogen, muscle and plasma lactate, hematocrit, and oxygen consumption) to support (or contradict) our behaviourally-based inferences related to the relationship between exercise intensity and metabolism. Behavioural indices and gait transition dynamics suggested that mean swimming speeds up to about 75 cm/s were maintained exclusively through aerobic metabolism, while those in excess of approximately 125 cm/s were fuelled by anaerobic processes. Data also suggested that a mixture of oxidative and glycolytic metabolism may have been used to support swimming speeds between 75 and 125 cm/s. This interpretation was supported by most of the physiological parameters measured, indicating that (1) a strong link exists between behaviour and exercise physiology within this species, (2) that behavioural observations of intact animals can provide accurate insights into physiological condition, and (3) suppression or alteration of behaviour may influence physiological measurements and the applicability of these data to fish under natural conditions.

A3.11 Dietary supplemental tryptophan increases plasma melatonin of a gastro-intestinal tract origin in rainbow trout: possible effect on aggressive behaviour

O. Lepage (Dept. Comparative Physiology, Evolutionary Biology Centre, Uppsala University), I. Meyer (Dept. of Fisheries and Marine Biology, High Technology Centre, Bergen, Norway), E. Larson (Dept. of Biology, Northeastern University, Boston) and S. Winberg (Dept. Comparative Physiology, Evolutionary Biology Centre, Uppsala University)

Elevated dietary intake of the amino acid L-tryptophan (TRP) results in a suppression of aggressive behaviour in rainbow trout (*Oncorhynchus mykiss*), an effect that could be related to elevated brain serotonergic activity, TRP being the precursor of serotonin (5-HT). However, 5-HT in turn is the precursor of melatonin, a hormone that may also have effects on aggressive behaviour. In this experiment we found that elevated dietary intake of TRP resulted in a drastic increase in daytime plasma levels of melatonin, an effect that was abolished if the fish were stressed. However, administration of an α-receptor antagonist prior to stress exposure prevented this stress-induced decline of plasma melatonin in TRP treated fish. These results clearly suggest that elevated daytime plasma melatonin levels in TRP treated fish is of gastrointestinal origin. This suggestion is further supported by the fact that melatonin content and secretion of gastrointestinal tissue incubated *in vitro* increased with increasing TRP concentrations of the incubation media. As in previous studies, elevated dietary TRP resulted in a suppression of aggressive behaviour. Treatment with citalopram, a selective 5-HT re-uptake inhibitor, (through the feed) had a similar effect, inhibiting of aggressive behaviour, whereas melatonin (i.p. implant) administration had no effect on aggressive behaviour. Thus, the effects of dietary TRP on aggression appear to be mediated by the brain 5-HT system and not by effects on melatonin synthesis and secretion.

A3.12 The role of brain monoamines and cortisol in control of stress responses and agonistic behaviour in fish

S. Winberg (Dept. Comparative Physiology, Evolutionary Biology Centre, Uppsala University), J. Schjolden (Dept. Comparative Physiology, Evolutionary Biology Centre, Uppsala University), T. Backström (Dept. Comparative Physiology, Evolutionary Biology Centre, Uppsala University), Ø. Øverli (Division of General Physiology, Department of Biology, University of Oslo), T.G. Pottinger (NERC, Centre for Ecology and Hydrology, Windermere Laboratory)

In rainbow trout (*Oncorhynchus mykiss*) the magnitude of the cortisol response to stress shows a high degree of heritability, and high responding (HR) and low responding (LR) lines of rainbow trout have been generated through selective breeding. In addition to divergent post-stress plasma levels of cortisol, HR and LR trout also differ in other neuroendocrine parameters. LR fish show higher brain serotonin turnover, and respond to acute stress with a larger increase in plasma catecholamines, than HR fish do. Moreover, a link between neuroendocrine stress responses and behavioural traits is evident. In particular, LR fish are socially dominant over HR fish when paired. LR trout also resume feeding faster than HR trout when transferred to social isolation, and display a reduced locomotor response in a territorial intrusion test. The combination of these results suggests that LR and HR trout are displaying divergent stress coping styles. A reciprocal relationship between the brain and endocrine system appear to exist, linking cortisol levels during stress to behavioural traits such as the outcome of agonistic interactions. The brain 5-HT system could provide such a link between stress responses and behaviour. Social subordination results in a drastic but reversible inhibition of behavioural responsiveness along with a sustained activation of the brain 5-HT system. However, cortisol appears to have time- and context-dependent effects on behaviour, which could either be mediated by interaction with 5-HT or other neurotransmitter systems, or reflect a direct behavioural role for cortisol receptors in fish.

A3.13 Using transcriptomics to profile rank position within dominance hierarchies: An SSH strategy

L.U. Sneddon (University of Liverpool), D.R. Williams (University of Liverpool), P. Koldkjær (University of Liverpool), J. Margareto (University of Liverpool), T.G. Pottinger (NERC Centre for Ecology and Hydrology, Windermere) and A.R. Cossins (University of Liverpool)

Functional approaches have been successful in elucidating the role of behaviour as a component of fitness, but it is now evident that a full understanding of how natural selection acts on such behavioural traits requires information about the mechanisms that cause them. Competitive interactions between conspecifics contributes to individual fitness and survival and is of fundamental theoretical interest. Many animals form dominance hierarchies or pecking orders where the dominant individual obtains exclusive or priority access to resources. Other studies have shown profound behavioural and physiological differences between individuals with differing dominance status. At present there is no information on the fundamental mechanisms governing position within a dominance rank, therefore, we have investigated the gene expression differences between rank members in the dominance hierarchies of rainbow trout (*Oncorhynchus mykiss*). This study aimed to understand the consequences of dominance behaviour by using innovative microarray technology to detect changes at the molecular level and correlate these to the behavioural output of an individual. Two libraries were constructed using Suppression Subtraction Hybridisation (SSH) that were enriched for differentially expressed genes. One library was constructed within the EC Stressgenes programme and subtracted between control animals and trout that were subject to abiotic stress and the other library was constructed by subtracting between dominant, subdominant and subordinate individuals. Comparison of data obtained using the two libraries will be discussed.

A3.14 The effect of cortisol on social status and brain monoaminergic activity in freshwater rainbow trout (*Oncorhynchus mykiss*)

J.D. DiBattista (Carleton University), H. Anisman (Carleton University), M. Whitehead (Carleton University), and K.M. Gilmour (Carleton University)

The main objective of this study was to determine whether elevated levels of circulating corticosteroids have the ability to influence the outcome of social interactions in salmonid fish. Juvenile freshwater rainbow trout, *Oncorhynchus mykiss*, were injected intraperito-

neally with cortisol and sampled after five days of social interactions with either similar sized or smaller conspecifics. In a separate experiment, small groups of fish were fed cortisol treated food for a period of five days, after which time their levels of brain monoamines and major metabolites ([5-HT] and [5-HIAA]; [DA] and [DOPAC]) were quantified using HPLC. In response to i.p. cortisol injections, experimentally-elevated plasma cortisol levels reduced the probability of larger fish within each pair preferentially becoming dominant, where large fish became dominant in 86% of the control pairs compared to only 45% in the cortisol treated pairs. Similarly, within size-matched pairs of fish, cortisol treatment significantly decreased the probability that the injected subject within each pair became dominant. Finally, cortisol administration via food pellets in the groups of unstressed fish was inversely correlated with dopaminergic activity in the telencephalon and brainstem regions as well as serotonergic activity within the hypothalamus. Therefore, based on our results, the effects of cortisol on social status in salmonids is most likely mediated via the modulation of central signalling systems rather than by extensive changes in physiological condition brought on by cortisol itself.

A3.15 Does serotonin inhibit aggression?

C.H. Summers (Biology and Neuroscience, University of South Dakota, USA)

Although neuromodulators like AVT/AVP, corticosterone, testosterone and nitric oxide may potently influence aggression, many studies suggest that serotonin underlies the basic inhibitory control of aggressive behavior and intent. However, serotonin is also purported to be the primary neurotransmitter system regulating behavioural and neuroendocrine characteristics of mood and stress. The theories used to explain these three behavioural states are not compatible. To understand the role of serotonin in regulating aggressive displays and acts, it is necessary to identify the scale of serotonergic release and putative sites of action, especially considering the limited number of serotonin producing cells in the brainstem, but widespread distribution of fibres and terminals. Regionally and temporally specific serotonergic action has been demonstrated in the lizard *Anolis carolinensis*, and a number of other vertebrates. In *A. carolinensis* temporally bimodal serotonergic activity is stimulated by social interaction and aggression, with the chronic phase both advanced and foreshortened in dominant males. In addition, activity of serotonergic terminals in limbic regions like hippocampus and amygdala are out of phase. In as much as serotonergic activity is expressed in a region specific manner, stimulated by aggressive activity, it is questionable whether serotonin directly inhibits aggression. It is also necessary to ask whether serotonergic regulation of aggression is active or passive. In addition, the question of regionally specific serotonergic inhibition of aggression must be addressed in the context of effects on mood and stress. It is clear that serotonin affects aggression, but it is not clear whether there is a causal negative relationship.

A3.16 Mobilising for war: use of energy reserves in fighting hermit crabs

M. Briffa (School of Biology and Biochemistry, Queen's University Belfast) and R.W. Elwood (School of Biology and Biochemistry, Queen's University Belfast)

When animals engage in fights they face a series of decisions, which are based on the value of the contested resource and either their relative or absolute fighting ability. Certain correlates of fighting ability or'resource holding potential' (RHP) such as body size are fixed but physiological correlates are expected to vary during the encounter. Here we examine the role of energy reserves and lactic acid levels in determining fight outcomes and fight vigour during'shell fighting' in hermit crabs. During these fights, the two contestants perform very different roles of attacker and defender. Attackers perform vigorous bouts of'shell rapping' whereas defenders remain withdrawn into their shells. First, we assessed the relationships between these metabolites and fight decisions by analysis of the physiological states of fighting animals at the end of contests. Physiological states at particular points in the contest, however, should also influence behaviour but this is difficult to assess by analysis of naturally concluding contests. The clear bouts of activity enabled us to analyse energy stores from animals that had been stopped from fighting at different points in the encounter. We show that in defenders glycogen reserves may be mobilised early, apparently dependent on their perceived likelihood of victory but any behavioural decision to'give up' comes later in the contest. Attackers, on the other hand, appear to be increasingly constrained by accumulating lactate and they give up when this crosses a threshold.

A3.17 Does pulsatile urea excretion serve as a social signal in the gulf toadfish, *Opsanus beta*?

K.A. Sloman (Brunel University), M.D. McDonald (University of Miami), J. Barimo (University of Miami), C.M. Wood (McMaster University) and P. Walsh (University of Miami)

Unlike many other teleosts, toadfish have a complete ornithine-urea cycle and can produce urea as a major nitrogen waste product. In some lab situations, toadfish release urea in large daily pulses, which can last for up to several hours. Urea excretion has also been docu-

mented in the field. The physiological mechanisms of pulsatile urea excretion in toadfish have received a lot of attention but little is known as to *why* toadfish pulse urea. We evaluated the hypothesis that the pulsatile excretion of urea by toadfish could serve as a social signal. Interactions between pairs of toadfish resulted in the formation of dominance hierarchies. In a first experiment, physiological parameters were measured in dominant and subordinate toadfish. In a second experiment, one fish of a pair was injected with ^{14}C-urea and the occurrence of urea pulses during social encounters documented. Behavioural activity did not influence the timing of pulse events and there was no effect of social status on pulse size. There was no correlation between pulsing and time of day but toadfish were generally more active at night. In a final experiment, the response of toadfish to 100 mM synthetic urea or the urea pulse excreted by another toadfish was observed. There was a tendency for toadfish to avoid synthetic urea but there was no apparent behavioural response to water containing toadfish urea. It appears that the pulsatile nature of urea excretion in the gulf toadfish does not function primarily as a social signal as previously hypothesised. However, it remains possible that the pulse event conveys information among fish that does not result in observable behavioural changes.

A3.18 Social modulation of androgen levels in vertebrates: a comparative approach using cichlid fishes

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Social modulation of androgens has been interpreted as an adaptation for the individuals to adjust their agonistic motivation according to a variable social environment, a hypothesis proposed by Wingfield and co-workers and known as the challenge hypothesis. It predicts that above a breeding baseline, androgen levels should reflect the social challenges an individual is exposed to. Therefore, higher androgen responsiveness (AR) to social challenges are predicted in males from monogamous species, that usually invest in parental duties, than in males of polygynous species, with low or no paternal care. In this study we tested the predictions of the ''challenge hypothesis'' in Cichlid fish, since a large variation in mating systems is present within this family. To control for phylogenetic bias, pairs of species that were closely related, but with varying levels of male–male interactions and paternal care were selected. For each species AR was first assessed to the interaction with an ovulating female, and secondly to additional challenges with a conspecific male intruder. Androgen levels (i.e. 11-ketotestosterone and testosterone) were measured from fish-holding water, to avoid invasive methods. Pair-wise comparisons show that the AR was higher in the monogamous than in the polygynous species, and that among polygynous species AR was higher in temporally pair forming species than in lekking species. These results provide further experimental evidence that supports the predictions of the CH in non-avian vertebrates, and they also suggest an effect of the degree of pair-bonding on the AR.

A3.19 Maternal effects and early development; linking mechanisms and trade-offs

P. Monaghan (Environmental and Evolutionary Biology, University of Glasgow)

In many species, females appear to be able to tailor the phenotypic development of their offspring to suit prevailing environmental circumstances, thereby enhancing offspring fitness. In birds, the composition of the egg, and other aspects of early nutrition, have very important consequences for the offspring. Female birds can alter the size, nutrient and hormone content, and the sex ratio of the eggs that they lay in apparently adaptive ways. This will influence the growth and development patterns of the young, and the competitive hierarchy within the nest. The pattern of egg investment has consequences for both parents and offspring and thus there is a need to balance the costs and benefits to both of changes in particular egg components. In this talk I will discuss experimental work on the effect of maternal condition on egg production patterns, highlighting the value of combining studies of underlying mechanisms with studies of fitness consequences.

A3.20 The long-term behavioural and physiological effects of variation in juvenile growth rates

N.B. Metcalfe (University of Glasgow)

It is taken for granted that adverse conditions early in life have immediate and negative effects on growth and early survival. However, the longer-term consequences of periods of poor nutrition are only just becoming evident. In this presentation, I will show that the interactions between the quality of the environment, the early growth of the individual and its adult performance are complex but intriguing.

Many animals have been shown to be capable of compensating for a period of low food availability by accelerating their rate of growth (above that of continuously fed controls) once food again becomes available, so that they partially or fully recover from the deficit. This indicates that growth rates are not normally maximised. This response is to be expected where there is a finite growth period, after which growth is no longer possible (so animals must accelerate if they are to make up for'lost' growth); more curiously, it also occurs in species such as fish that have indeterminate growth patterns.
While the benefits of catching up to reach a'normal' adult size are obvious, there is recent evidence that this rapid growth is also costly. In this talk I will describe the types of cost, which can be categorised both in terms of timescale (e.g. immediate vs long-term costs) and type (behavioural, physiological, molecular), and will show how costs range from an increased risk of being predated through to an accelerated rate of senescence.

A3.21 Behavioural consequences of compensatory resource allocation during growth

N.J. Royle (University of Glasgow, Scotland), J.E. Lindström (University of Glasgow, Scotland) and N.B. Metcalfe (University of Glasgow, Scotland)

Poor conditions during early growth may have long-lasting consequences on an individual's later behaviour and performance. This may be counteracted by compensatory mechanisms, such as 'catch-up' growth – if conditions improve an individual can accelerate growth and re-gain the normal growth trajectory. However, this compensation appears to carry costs: recent studies have revealed a variety of costs from impaired physiological function to reduced lifespan. We present the results from experiments to assess the costs and benefits of compensatory growth in relation to sexual selection in a model species, the green swordtail (*Xiphophorus helleri*). At maturity male swordtails develop an ornamental extension of the caudal fin, the 'sword'. Recent studies have shown that females prefer larger bodied males, but after controlling for body size show a strong preference for males with longer swords. The effect of the sword is to increase the apparent size of the male at relatively low cost compared to a similar increase in body size. However, there are also potential costs of not investing in body size, as larger males tend to be dominant over smaller-bodied males. A disproportionately long sword may also impair both burst and sustained swimming performance, whilst there may be costs associated with fast growth *per se*. We examined these trade-offs in compensatory resource allocation strategies through experimental modification of dietary regimes and then measured components of fitness, such as swimming performance, attractiveness, dominance and survival in relation to nutritional conditions during development.

A3.22 Abstract not supplied

A3.23To what extent is foraging behaviour of aquatic birds constrained by their physiology?

P.J. Butler (School of Biosciences, University of Birmingham), J.A. Green (School of Biosciences, University of Birmingham) and L.G. Halsey (School of Biosciences, University of Birmingham)

Aquatic birds have access to a limited amount of usable O_2 when they forage (dive) underwater, so the major constraint is the need periodically to visit the water surface to replenish these stores and remove accumulated CO_2. The size and the rate at which the O_2 stores are used ($\dot{V}O_2$) are the ultimate determinants of the duration that aquatic birds can remain underwater at the food source, without resorting to anaerobic metabolism. However, the assumption that the decision to terminate a dive is governed solely by the level of a bird's O_2 stores is not always valid. The $\dot{V}O_2$of tufted ducks during diving is about 3 times the resting value, but they dive well within their calculated aerobic dive limit (cADL, total usable O_2 stores/$\dot{V}O_2$during diving). Furthermore, the rates of CO_2 production during dives and removal during surface periods, seem to influence the duck's foraging behaviour. Nevertheless, behaviour still consistent with the predictions of diving models based on the balancing of oxygen stores during the dive cycle. Some species, such as macaroni and emperor penguins, dive for durations closer to their cADL and may organize their diving to maximize the proportion of a diving bout spent at the food source. In contrast, many species, such as gentoo and king penguins, appear to remain submerged for excessive durations, with about 20% of their dives exceeding the cADL, even if $\dot{V}O_2$during diving is assumed to be the same as that of birds resting in water.

A3.24 Stable isotopes: a tool for linking behaviour and physiology

R.M. Bevan (School of Biology, King George VI Building, University of Newcastle)

Behavioural ecologists rarely interpret their data in the context of an animal's physiology and physiological capabilities. Similarly, the physiological responses of animals in the laboratory can be meaningless unless linked with the natural behaviour of the study animal. By integrating the two, it is possible to gain a greater understanding of the overall ecology of an animal. Stable isotopes offer a potential means of bridging the two disciplines.

For example, the doubly labelled water technique uses ^{2}H and ^{18}O to estimate the metabolic rate and water turnover of animals and is a technique that is widely used by field ecologists. Thus, artificially elevated levels of stable isotopes have been routinely used as biotracers by animal ecophysiologists.
However, biologists are increasingly using the natural abundance of stable isotopes, to investigate aspects of animal ecology. This has been due to recent developments in mass spectrometry. Stable isotopes can be used to reconstruct the diet of an animal *e.g.* recent data imply that Arctic terns and Atlantic puffins, while feeding on the same food source, occupy different trophic levels suggesting that we are underestimating the role of other prey in their diet. Stable isotopes are also being used to monitor the movements e.g. migration, of birds that could not be tracked by more conventional techniques. Other potential uses of stable isotopes include determining tissue turnover and nutrient allocation in free-living animals. Thus, stable isotope analysis is proving to be an important technique for gaining both behavioural and physiological information from animals.

A3.25 Reciprocal interactions between behaviour and respiration

R. Stephenson (Dept of Zoology, University of Toronto, Canada)

The alternation between states of sleep and wakefulness, and between diving and surfacing represent well-studied examples of situations in which behaviour and respiration oscillate in a functionally integrated way. However the approaches taken to understand the functional relationships have traditionally been very different in the fields of sleep and diving. Sleep researchers have focussed on physiological mechanisms that mediate sleep-related respiratory variability, whereas in the diving field the main approach has been to develop optimal foraging models to understand the adaptive significance of dive patterns. I will attempt to bridge the two fields by exploiting and adapting the concepts developed to explain sleep apnoea, to propose a model for the physiological control of diving behaviour in aquatic mammals. Computer simulations, based on a mathematical model of the mammalian respiratory control system, suggest that the temporal organisation of routine diving activities of Weddell seals may be determined by the dynamic characteristics of the cardiorespiratory system. It is suggested that a diving seal is stimulated to begin its ascent to the water surface at the end of a dive by any positive value of respiratory drive; i.e. that apnoea is maintained by disfacilitation, rather than inhibition, of respiratory drive. This ''respiratory resonance model'' postulates that routine diving behaviour is tuned to the dynamic characteristics of the respiratory control system. Simulations show that the dynamics of the respiratory control system can be modulated by both behavioural and physiological adjustments indicating a close reciprocal interaction between respiratory and behavioural regulation. Supported by NSERC Canada.

A3.26 The effect of hypoxia on the behavioural and physiological thresholds of Atlantic cod

N.A. Herbert (University of Copenhagen) and J.F. Steffensen (University of Copenhagen)

Atlantic cod, *Gadus morhua*, were exposed to a progressive stepwise, decline in water oxygen level (95, 65, 50, 40, 30 and 20% O_2 saturation) over a 7 hour (1000 h–1700 h) experimental period. Behavioural response thresholds, in terms of swimming speed ($BL.s^{-1}$) and direction, were measured remotely using a customised computer-video tracking system and compared with background routine activities (measured over the same period of time but on a previous day). Physiological response thresholds were measured by an abrupt shift in plasma cortisol, glucose, osmolality and blood lactate and were compared with behavioural alterations during the hypoxic event.Both background and hypoxic swimming speeds were reduced gradually over the 7 h observation period. However, repeated measures ANOVA revealed a clear behavioural threshold during hypoxia; cod swimming speeds were reduced significantly at 40–20% O_2 saturation. Cod did not exhibit any preference for the direction of swimming and this was not adjusted by hypoxia. Plasma glucose remained unadjusted throughout all levels of hypoxia. Plasma cortisol and blood lactate were not significantly elevated until a level of 20% O_2 saturation. Plasma osmolality exhibited a transient rise at 50% saturation but decreased back to control levels between 40–20% saturation. The results indicate that the behavioural response of cod to hypoxia is not triggered by severe respiratory distress.

A.27 Effects of perceived risk of predation on reflex responses to hypoxia in the flathead mullet (*Mugil cephalus*)

A. Shingles (IMC, Italy), D.J. McKenzie (CNRS-IFREMER, France), G. Claireaux (CNRS-IFREMER) and P. Domenici (IMC, Italy)

The flathead mullet inhabits frequently hypoxic and turbid estuaries and coastal lagoons. In hypoxia, mullet perform aquatic surface respiration (ASR), skimming well-oxygenated surface waters. This behaviour, however, increases risks of avian predation. We investigated the role of O_2-sensitive chemoreceptors in stimulating the ASR response; whether ASR behaviour was modulated by visual exposure to a model avian predator, and

how water turbidity influenced effects of the ''predator''. Chemoreceptors were stimulated by boluses of sodium cyanide (NaCN) infused into the ventilatory water stream (''external'') or into the bloodstream (''internal'', via arterial catheter) of mullet in aquaria with surface access. Both interventions stimulated reflex cardioventilatory responses similar to those elicited by hypoxia, namely bradycardia, gill hyperventilation, and ASR. Therefore, ASR is stimulated by chemoreceptors sensitive both to environmental and systemic O_2 levels. The model predator elicited bradycardia and gill hypoventilation. External NaCN then caused further bradycardia but had little effect on gill ventilation. The lag to initiation of ASR reflexes was increased from 35 s to 120 s by the ''predator'', but response intensity (number of events, time at the surface) was unchanged. However, fish usually surfaced at the aquarium edges or under a covered area at the rear. Therefore, perceived risk of predation influenced cardiac, branchial and ASR reflexes differently, and significantly modulated ASR behaviour. Turbid water (300 NTU Kaolin) abolished responses to the predator, but mullet usually performed ASR under cover. Thus, mullet reduce risks of predation during hypoxia and in turbid waters by searching for sheltered areas to perform ASR.

A3.28 Behaviour and physiology of fish exposed to hypoxia

J.F. Steffensen (Marine Biological Laboratory, University of Copenhagen)

How can we monitor the behaviour of fish? Recently developed real-time computerized-video tracking software, with optional feed-back depending on the behaviour of the fish, will be described – and demonstrated.
Laboratory experiments with somewhat similar equipment showed that the voluntary routine swimming activity of single fish (i.e. Atlantic cod) exposed to hypoxia decreased with progressive hypoxia. Maximum possible swimming speed also decreased with decreasing oxygen availability. This was shown by experiments carried out in swimming respirometers (i.e. rainbow trout).
Small schools of herring exposed to hypoxia in laboratory conditions either maintained or increased swimming speed in response to moderate hypoxia, depending on the initial swimming speed. At certain low oxygen level, however, oxygen availability became limiting and the swimming speed decreased dramatically. At a continued decline in oxygen the schools even disintegrated.
A model of oxygen consumption of swimming fish, based on swimming respirometry of single fish, and calculated oxygen availability within a school, support this observed behaviour. From the model maximum school size can be calculated for different swimming speeds and oxygen levels.
In the near future we plan to measure oxygen levels within fish schools in the field. We wish to check to what extent they do become hypoxic and if the level corresponds to the predicted values. According to the hypothesis environmental hypoxia will result in smaller fish schools that will benefit from swimming at speeds close to the optimal.

A3.29 Population differences in response to hypoxia: behaviour and physiology of 3-spined sticklebacks

E. O'Connor (Liverpool), T.G. Pottinger (CEH, Windermere), A.R. Cossins (Liverpool) and L.U. Sneddon (Liverpool)

Perturbation of the natural environment can deleteriously affect both the behaviour and physiology of animals, leading to a suite of responses some of which may be adaptive. One environmental factor of particular importance is reduced environmental PO_2 which by constraining metabolism may limit energetically expensive behaviours. One such behaviour is aggression, which is crucial in the maintenance of dominance hierarchies in fish communities. Fish chronically exposed to different levels of environmental hypoxia during development or over many generations may better tolerate hypoxia thereby maintaining aggressive behaviour and dominance hierarchies. This prediction has been tested by comparing populations of 3-spined stickleback (*Gasterosteus aculeatus*) from different natural habitats such as static ponds and flowing rivers. Fish were exposed either to hypoxia (20% oxygen saturation) or normoxia and dominance ranks constructed by monitoring aggressive interactions and feeding rates. The fish were killed and L-lactate, glucose and cortisol assays were performed on whole body homogenates to record physiological responses. In order to calibrate the level of stress activated by hypoxia the same physiological parameters were measured after a standard stressor-confinement. Results for populations from different habitat types will be compared and the differences between the population's response to environmental stress discussed.

A3.30 Habitat choice of Atlantic cod (*Gadus morhua*) under the influence of hypoxia

J.E. Skjæraasen (Department of Fisheries and Marine Biology, University of Bergen) and A.G.V. Salvanes (Department of Fisheries and Marine Biology, University of Bergen)

The Atlantic cod is a demersal temperate water teleost distibuted on coastal shelves on both sides of the Atlantic. Within these areas individual cod experience various

oxygen and temperature regimes. Individual fish may thus have to cope with short periods of hypoxia (e.g. in coastal areas and fjords) or live under permanent hypoxic conditions (e.g. in the Baltic Sea). Differences between genotypes in their ability to cope with hypoxic stress may therefore influence individual behaviour, survival and on larger scales-population structure. For cod the main oxygen carrier in the blood is hemoglobin. Adult cod have a two allele hemoglobin system, which results in three different genotypes; two different homozygotes and a heterozygote. These genotypes have different temperature optimums and affinites for oxygen. Large differences in hemoglobin allele frequencies have also been found between populations. We present results from experiments where cod with different hemoglobin genotypes could choose freely between habitats with different oxygen contents. Results will be discussed in relation to functional properties of hemoglobin, and population structure.

A3.31 Predicting social dominance

W.J. Korzan, (University of South Dakota), Ø. Øverli (University of Oslo), G.L. Forster (University of South Dakota), M.J. Watt (University of South Dakota), E. HÖglund (University of Oslo), Cliff H. Summers (University of South Dakota)

In *Anolis carolinensis*, eyespot formation, i.e. darkening of postorbital skin from green to black, is stimulated by sympathetic activation of β_2-adrenergic receptors. Eyespots form more rapidly in dominant males during social interaction, and aggressive behavior is reduced when animals see eyespots on an opponent. To assess the effect of eyespots on central monoamines during social interaction, males were paired by weight. They were painted postorbitally with green or black paint and allowed to interact for ten minutes, males with artificially darkened eyespots paired with males that had hidden eyespots. All males that viewed an opponent with black painted eyespots became subordinate. In these subordinate animals, dopamine and DOPAC was elevated in the medial amygdala. In contrast, males that viewed opponents with hidden eyespots and became dominant had increased dopamine in the nucleus accumbens, paleostriatum and substantia nigra/ventral tegmental area. Recent work on behavior after a novel stressor, other than aggression (i.e. feeding) may provide clues in predicting social rank. The role of dopamine systems in the motivation to initiate these behaviors is well established. These results suggest ample contextual flexibility in central dopaminergic systems, sufficient for an animal to initiate behavior and establish dominance or subordination, with activity also influenced by the formation of this social hierarchy. For example, dopamine release in nucleus accumbens maybe involved in motivation to elicit the behavior required to achieve dominant status. Furthermore, social status and central dopaminergic activity was determined by a visual cue, the presence or absence of postorbital eyespots on an opponent.

A3.32 The influence of social status on the erythrocyte adrenergic stress response in rainbow trout (*Oncorhynchus mykiss*)

J.B. Thomas (Carleton University, Ottawa), S.F. Perry (University of Ottawa) and K.M. Gilmour (Carleton University, Ottawa)

In the present experiment we investigated whether social status within rainbow trout dominance hierarchies influences the erythrocyte β-adrenergic stress response. Social status of individual fish within pairs of size-matched conspecifics was determined by behavioural observations. Utilising real-time PCR we measured the expression of erythrocyte β-3b adrenergic receptors, as well as the β-3b receptor linked sodium-hydrogen exchanger (β-NHE). It was found that following acute (<48 hours) and chronic (approximately 1 week) social interaction that subordinate rainbow trout generally demonstrated greater expression of both β-3b receptor and β-NHE mRNA. Additionally, erythrocyte swelling and pH responses to isoproterenol were used as indices of erythrocyte adrenergic responsiveness. Erythrocyte swelling did not significantly differ between dominants and subordinates in acute pairings whereas in chronic pairings, subordinate fish demonstrated elevated erythrocyte swelling under all isoproterenol doses. However, despite subordinates demonstrating greater erythrocyte swelling in chronic pairings, the magnitude of the response was not significantly impacted by social status. Plasma pH did not significantly differ between dominants and subordinates in either acute or chronic pairings. The results of the study suggest that social status within rainbow trout dominance hierarchies does not significantly impact the erythrocyte β-adrenergic stress response.

A3.33 Renal morphology of the euryhaline flounder (*Platichthys flesus*)

E. Weybourne (School of Biological Sciences, University of Manchester), J.M. Warne (School of Biological Sciences, University of Manchester), R.J. Balment (School of Biological Sciences, University of Manchester)

Considerable variation in the renal nephron structure is seen in teleost fish. In this study light microscopy has been used to describe the organisation of the nephron in the euryhaline flounder (*Platichthys flesus*). Renal tissue was fixed using paraformaldehyde or Bouins and wax

embedded. 5μm thick sections were cut and stained using haematoxylin and eosin and then examined by light microscopy. It was found that the nephron of the flounder was composed of a prominent renal corpuscle and a renal tubule made up of four distinct segments. A neck segment was found to be present between the glomerulus and proximal tubule. The proximal segment bearing a distinct dense 'brush border' made up around 90% of the tubular mass. A short distal segment was present followed by a collecting segment characterised by large diameter and smooth muscle surround, emptying into the large collecting duct system. The presence of the distal segment maybe important in the ability of flounder, a primarily marine species, to adapt to hypoosmotic media, as this segment is not present in fully marine pleuronectids (Hentschel and Elger, 1987).

Hentschel, H., & Elger, M., (1987). The distal nephron of the kidney of Fishes – Introduction. *Advances in Anatomy, Embryology and Cell biology* 108:1–146.

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A3.34 Post-prandial oxygen consumption in the Dover Sole (*Solea solea*)

M. Cannas (IMC, Italy), G. Claireaux (C.R. É.M.A.-L'HOUMEAU, France), P. Domenici (IMC, Italy)

The energy available by the fish is influenced by environmental variables (such as temperature, oxygen, salinity) which are involved in setting the limits within which internal bioenergetic processes take place. The biological consequences affect fish performance e.g. survivor, swimming, growth, reproduction, or SDA (i.e. Specific Dynamic Activity, the metabolic cost of ingestion, digestion and food absorption).

The aim of these experiments was to evaluate the effect of the meal size on oxygen consumption and duration of the digestion and to relate them with metabolic scope. We tested the oxygen consumption variations in the sole after feeding them with 1% to 2% of the body weight using live worms.

Preliminary results show that after feeding, the sole oxygen consumption increases progressively and attains a peak four hours later. This peak is similar to the sole active metabolic rate (maximum oxygen consumption recorded during sustained activity). Our results also suggest that meal size has no effect on the level of oxygen consumption during digestion, but has an effect on the duration of SDA.

A3.35 Expression of inducible stress protein HSP70 in the tissues of *Puntius filamentosus* exposed to conspecific alarm substance

A. Paglianti (Università di Firenze, Firenze Italy), F. Ceccolini (Università di Firenze, Firenze Italy), L. Colli (Università di Parma, Parma Italy), J. Tagliavini (Università di Parma, Parma Italy) and R. Berti (Università di Firenze, Firenze Italy)

When predators mechanically damage the skin of ostariophysan fish an alarm substance is released from epidermic club cells. Nearby conspecifics or individuals of closely related species detect these chemicals by olfaction and perform a species-specific fright reaction. Such fright reactions include schooling, hiding, stereotyped swimming patterns and have been assumed to reduce risk of predation. Fish may be stressed when they detect alarm substance, an index of predator presence, since psychological stimuli lead to changes at the molecular and biochemical levels. Perturbations in the physicochemical environment that act as stressors in fish result in induction of stress proteins. The aim of this work was to detect and quantify, by Western blotting and anti-HSP70 monoclonal antibody, the inducible protein in brains, muscular, and gills tissues of *Puntius filamentosus* (Cyprinidae) stressed by alarm substance.

Preliminary results suggest that alarm substance that induces the fright reaction, also induces HSP70 both in brain and in muscle tissues.

A3.36 Stress coping styles in juvenile brown trout (*Salmo trutta*)

D. Brelin (Evolutionary Biology Centre, Uppsala University, Sweden), E. Petersson (Institute of Freshwater Research, The Swedish Board of Fisheries), S. Winberg (Evolutionary Biology Centre, Uppsala University, Sweden)

Various animal studies, predominantly in rodents and birds, have stated the existence of two distinct coping styles, or ''personality types'', within populations. The two types of animals are characterized as either displaying a proactive or a reactive coping style. Each coping style is associated with a set of defined behavioural and physiological characteristics. In a given situation, proactive animals show more aggression, a higher general activity and a predominant sympathetic activation. Contrary, animals with a reactive coping style respond with more immobility and lack of initiative, and a predominant parasympathetic/hypothalamic activation. The presence of divergent coping styles has been indicated in several studies on salmonid fish. However, in these studies only one, or in some cases a few parameters have

been examined. In this study we describe and evaluate a method for distinguishing these two coping styles in a brown trout population. We used a series of tests on individual juvenile trout, each test including a common key aspect of the two different coping styles. The tests conducted and the parameters measured were as follows: 1) Recovery of feeding following transfer to experimental tanks; individual daily food intake quantified. 2) Aggression; latency to first attack and the number of attacks performed during the following 30 minutes quantified in a resident-intruder test. 3) Behavioural responses to hypoxia; escape attempts, movements and activity quantified from video recordings. 4) Endocrine stress responses; blood plasma cortisol and catecholamine levels quantified following 45 min confinement stress.

A3.37 Ultraviolet light and mate choice in the three-spined stickleback

P. Boulcott (University of Edinburgh), K. Walton (University of Stirling) and V. Braithwaite (University of Edinburgh)

The importance of vision and sign stimuli in the courtship rituals of the three-spined stickleback *Gasterosteus aculeatus* has been a central theme of much of the evolutionary debate concerning this important study species. Yet, despite the apparent importance of the stickleback's visual system, experimental investigation which examines the role that colour plays in these mate-choice decisions has typically employed techniques more appropriately applied to humans. In so doing, it is probable that by arbitrarily omitting ultraviolet wavelengths from their analysis, researchers have omitted a region of the electromagnetic spectrum to which the three-spined stickleback is suspected to be sensitive, and which could be involved in visual signalling. Such a notion is supported by the recent evidence suggesting that ultraviolet cues are used in the mate-choice decisions of other shallow-dwelling freshwater fish. We examined the mate-choice decisions of sexually receptive female sticklebacks under an experimental protocol where the ultraviolet content of two subject males was manipulated. In our series of experiments, two competing males were viewed by the female through either ultraviolet blocking or ultraviolet transmitting filters that possessed identical transmission characteristics across the human visible region of the spectrum. Our results revealed that males were found to significantly prefer males viewed under full spectrum conditions over those viewed through the ultraviolet blocking filter. The same manipulation of the visual signal was not, however, found to affect the shoaling behaviour of subject fish when viewing shoaling conspecifics through the same experimental set-up. Given this, our findings raise the possibility that ultraviolet wavelengths affect the mate-choice decisions of the three-spined stickleback and could contribute to the assessment of male fitness by females in this species.

A3.38 Stress and coping strategies in rainbow trout (*Oncorhynchus mykiss*) selected for high and low cortisol response to stress

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When confronted with a stressful situation, most animals will change their behaviour in order to reduce or eliminate the stressful stimuli. In several studies individual differentiation in behavioural and physiological responses to stress suggest that at least two different stress coping styles can be identified. At CEH, Windermere, UK two different selection lines of rainbow trout have been produced based on their cortisol response to a standardized stressor, resulting in high responsive (HR) and low responsive (LR) individuals. It has been shown that LR fish to a great extent obtain social dominance over HR fish when matched in pairs. This suggests that these two selection lines differ in behaviour in that the LR fish is more aggressive than the HR fish. A high level of aggression is often displayed by individuals that exhibit the proactive coping style, while the more passive individuals exhibit the reactive coping style. We wanted to find out if there was a difference in behaviour between the two selection lines and if they in fact represent two different coping styles. In our study we found that the HR fish attacked the intruder more, they were ''bolder'' in exploring new territory and more active on a general basis than the LR fish. At a first glance these results suggest that the behaviour displayed by HR fish in this study represents a proactive coping style, while the LR fish seem to be reactive. However, this is not necessarily the case, which will be discussed in detail at this talk.

A3.39 Brain areas activated in rainbow trout post stress

T. Backström (Evolutionary Biology Centre, Uppsala University), A. Nyström (Evolutionary Biology Centre, Uppsala University), S. Winberg (Evolutionary Biology Centre, Uppsala University)

Stress has various effects on animals, therefore studies of different aspects could provide a better understanding,

and regenerate new ways to prevent harmful effects. Gene activation post stress can be measured by *c-fos* gene induction. In our study we were interested in which areas in teleost brain were activated following exposure to a standardized stressor, and the development of activation over time.
Rainbow trout (*Oncorhynchus mykiss*) were put in confinement (stressor) for 0 (control group), 1, 2, 3 or 5 hours. Brain and blood samples were taken. Cortisol levels were analyzed from blood samples. Brain tissue was sectioned on a cryostat, and cells expressing *c-fos* were visualized by immunohistochemistry.Cortisol levels were elevated in fish that were confined 1, 2, 3 and 5 hours, as compared to controls.
c-fos induction was clearly seen in locus coerelus, preoptic area and raphe nuclei. The induction was increasing over time, but cell numbers increased most between 1 and 3 hours.
The areas concerned are going to be more thoroughly studied, to learn which substances are activated and their function. Further investigation is also planned to explore when activation peaks in the different areas.

A3.40 The production of chemical alarm signal and the behavioural response to its detection in the cave cyprinid *Phreatichthys andruzzii*

F. Ceccolini (Università di Firenze, Firenze Italy), A. Paglianti (Università di Firenze, Firenze Italy) and R. Berti (Università di Firenze, Firenze Italy).

Phreatichthys andruzzii is a cave fish endemic of a Somalian subterranean hydrographic system. Histological analysis of *P. andruzzii* epidermis reveals the presence of club cells responsible for production of alarm substance and *P. andruzzii* skin extract elicited alarm reaction in *Puntius filamentosus*, a phylogenetically related epigean species. Thus, there was no regression for alarm substance production due to subterranean life. For what concern behavioural reaction, *P. andruzzii* were filmed in IR light after introduction of conspecific alarm substance. We analysed spatial distribution considering distance from bottom, from releasing site, and from group baricenter. *Phreatichthys andruzzii* does not significantly respond to conspecific skin extract detection. Cannibalistic habits of this species could make *P. andruzzii* indifferent to conspecific alarm substance. On the contrary, when *P. andruzzii* were tested with eterospecific skin extract (*P. filamentosus*), we observed significant increase of the density of the shoal and an approach to the bottom during the first five minutes. Alarm behaviour in this species does not present patterns typical of epigean species, like zigzag swimming. Interpreting spatial alteration both as alarm reaction or as avoidance response, the reaction to an eterospecific alarm substance in *P. andruzzii* can be advantageous to avoid to cross the limits of the subterranean biotope, since *P. andruzzii* has no sympatric species.

A3.41 Seasonal differences in the diving behaviour of macaroni penguins: balancing physiological and ecological pressures

J.A. Green, A.J. Woakes (School of Biosciences, University of Birmingham), I.L. Boyd (Sea Mammal Research Unit, University of St Andrews) and P.J. Butler (School of Biosciences, University of Birmingham).

Previous studies of macaroni penguins diving during the breeding season to feed and provision their chick on the island of South Georgia showed that nearly all diving was aerobic in nature. Very few dives exceeded the calculated aerobic dive limit (cADL), which is computed from the total body oxygen stores divided by the estimated rate of oxygen consumption while diving. Adjustments in circulation and heart rate by the penguins appeared to permit bouts of repeated dives with durations close to the cADL. However, recently collected data reveal that the behaviour of this species may be quite different during the long winter migration. At this time of year macaroni penguins move away from South Georgia and spend many months at sea foraging in areas different from those used during the breeding season. This change in foraging location appears to induce a change in diving behaviour, with the penguins performing many longer and deeper dives. We discuss the different ecological and physiological pressures on this species at different times of year and how these may manifest themselves in observed behaviour.

A3.42 Suppression of aggressive behavior in juvenile Atlantic cod (*Gadus morhua*), by L-tryptophan supplementation

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Aggressive interactions and cannibalism in juvenile Atlantic cod have been reported to cause big losses during intensive rearing conditions. However, very little is known about the social behaviour and neurochemistry underlying intraspecific competition in juvenile Atlantic

cod. In other teleosts, increased brain levels of serotonin are associated with suppressed aggression. For instance, dietary supplementation with the serotonin precursor, L-tryptophan, inhibits aggressive behaviour in rainbow trout. In the present study, pairs of juvenile Atlantic cod were allowed to interact in order to characterise aggressive behaviour. The following aggressive acts were observed: approach; a fish approaches an other fish with the mouth bottom and operculum dilated, nip/bite; a fish nips or bites the other, chase; an attacking fish follows the escaping fish for more than three body lengths. The behavioural effect of dietary L-tryptophan supplementation was observed in pairs subjected to repeated encounters for 10 days. Each day, pairs were allowed to interact until aggression was initiated, and then for an additional 10 minutes, whereupon the fish were re-isolated. On the third day the feed was exchanged from commercial fish feed to feed supplemented with 25 mg/g L-tryptophan (by dry weight). TRP treatment resulted in decreased aggressive behaviour compared with fish receiving feed without L-tryptophan supplementation, and increased central levels of serotonergic activity, quantified as the relative concentrations of the serotonin metabolite, 5-hydroxyindoleaectic acid, and serotonin. This study suggests that the juvenile Atlantic cod is highly aggressive and that suppression of this behaviour is associated with increased levels of serotonergic activity.

A3.43 Individual recognition in the lobster, *Homarus americanus*: The loser remembers

M.A. Steinbach (Boston University Marine Program, Woods Hole) and J. Atema (Boston University Marine Program, Woods Hole)

Lobsters can distinguish conspecifics individually by odor, and use this information, carried in the urine, to establish their social structure through a series of agonistic encounters. In the first encounter, unfamiliar lobsters learn the individual odortype of their opponents. In second and subsequent encounters, they do not engage in highly aggressive interactions. In this study, we questioned which of an agonistic pair makes the decision not to fight a second time. We paired male lobsters in two successive boxing matches. Before the second fight, we disabled the critical antennular chemoreceptors of either the winner or the loser of fight one. The effects of the lesion on the behavior of both animals as well as the overall characteristics of the fight were recorded. Results show that when the subordinate's chemoreceptors are disabled and the dominant remains intact, all behaviors and fight characteristics remain largely the same in fight two as in fight one. In two cases, the lesioned loser of fight one beat his dominant in fight two, thus overturning their dominance relationship. When the winner's nose is disabled and the loser remains intact, however, the duration of the fight and all other measures of aggression decrease significantly for both winner and loser. These findings confirm that the loser of a fight determines the intensity of subsequent fights, fleeing significantly sooner and more often, thereby eliciting less aggression from the winner.

A3.44 Tuning of mechanoreceptors in the lobster antennule

V. Miller-Sims (Boston University Marine Program, Woods Hole) and J. Atema (Boston University Marine Program, Woods Hole)

The American lobster, *Homarus americanus*, is capable of tracking an odor to its source in a turbulent plume. In such a plume an animal encounters hydrodynamic and chemical signals. Concurrent reception of odor and flow could be an important cue for odor plume tracking. The lateral antennule contains many chemoreceptor neurons and is important for successful tracking. We now want to determine the bandwidth in which the lobster uses lateral antennule mechanoreceptors to monitor the hydrodynamic properties of the plume. Under oscillatory flow conditions the antennule has a resonance of frequency of 5–12 Hz; antennular mechanoreceptors could also be expected to respond optimally in this range. In this experiment frequency synchronization is used to determine the types of hydrodynamic stimuli to which the antennule is capable of responding. The distal end of the antennule was fixed in a speaker driven oscillatory flow chamber to stimulate whole antennule movement. We recorded extracellular responses from antennular axons to oscillatory antennule movement over a frequency range of 1–128 Hz in an amplitude range of 16 to125 um. Responses were recorded from 30 cells and synchronization coefficients were computed for each stimulus presentation. As a population these cells were capable of synchronizing to the stimulus at frequencies between 4 and 64 Hz. Cells with low to no background spiking synchronized best at lower frequencies of 4–16 Hz while cells with high background spiking synchronized best at higher frequencies between 16 and 64 Hz. This frequency range may have significance in monitoring turbulent plume dynamics.

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