Presence of shelter reduces maintenance metabolism of juvenile salmon

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Summary

1. Shelter is of major importance to many animals in providing protection against both the physical environment and potential predators.

2. We hypothesized that animals without shelter suffer metabolic costs associated with a need for increased vigilance and preparedness to escape attacks from predators or competitors. This possibility was tested by comparing the standard metabolic rates of inactive postdigestive juvenile Atlantic salmon, *Salmo salar*, held either with or without a shelter, which took the form of a semicircular ledge under which the fish could fit comfortably.

3. The ledges were semitransparent (so did not substantially reduce light levels) and provided no protection against the minimal water velocities in the testing arena. Nonetheless, absence of ledge shelter resulted in a 30% higher rate of oxygen consumption.

4. Fish without a ledge shelter typically positioned themselves against vertical walls of the observation arena, which presumably afforded the best available sheltering option, and adopted a significantly darker coloration (indicative of greater stress) than those under ledges. Fish with ledges rested outside and adjacent to rather than beneath the shelter. Therefore, it seems that awareness that a shelter is readily available, rather than the act of sheltering, results in reduced metabolism.

5. We conclude that the presence of appropriate shelter not only reduces the risk of predation but also provides a metabolic benefit to fish that is likely to have implications for growth performance and activity budgets. Standard metabolic rate can be a function of habitat structure.

Key-words: Energy budgets, metabolic costs, *Salmo salar*, standard metabolic rate, vigilance

Introduction

The availability of shelter has been shown to be of extreme importance to the survival of animals. The reasons for using shelter are numerous, but they fall into two broad categories: protection against the physical environment and protection against other animals. The physical environment in which animals live can sometimes be very hostile. For example, many marine intertidal organisms have to tolerate periods of air exposure. Crabs are able to survive these dry periods by moving to suitable moist cracks, crevices or burrows that significantly reduce the risk of water loss (Nybakken 1993). Seebacher & Alford (2002) showed that it was essential for Cane Toads, *Bufo marinus*, to find suitable diurnal shelters in order to prevent dehydration and

temperature stress during the dry season. In the Brazilian Lizard, *Tropidurus torquatus*, the abundance of microhabitats suitable for egg development influences the length of the breeding season since low levels of moisture negatively affect egg survival and reduce embryo sizes (Wiederhecker, Pinto & Colli 2002). Significant energy economies may be accrued by sheltering from the environment. For example, sheltering can potentially reduce water loss by as much as 81% in the Hoopoe Lark, *Alaemon alaudipes* (Williams, Tieleman & Shobrak 1999) and can cut locomotory costs of fish holding station in flowing waters (Rimmer, Paim & Saunders 1984). Such energy savings can also have indirect benefits for reproduction: the use of nestboxes during the night by Great and Blue Tits during the egg-laying period has been suggested to reduce the birds' thermoregulatory costs (Pendlebury & Bryant 2005), and therefore allow an enhanced allocation of resources to egg production.

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Many animals use shelters to hide from predators (Kotler 1984; Godin 1997; Valdimarsson & Metcalfe 1998; Steele 1999), but the benefits may not be restricted to the obvious increase in safety. Shelters may replace the need for other mechanisms for avoiding predators, such as camouflage or vigilance, which may have associated costs, for example in producing appropriately coloured compounds and maintaining acutely active sensory systems. The absence of a refuge might be expected to result in an overall elevation in metabolism of the animal even when quiescent. However, we are not aware of any other study regarding the occurrence or magnitude of such additional energy costs in the absence of shelter.

The objective of this experiment was to evaluate whether the presence of a shelter reduces the standard metabolic costs. Standard metabolic rate (SMR) can be measured as the oxygen consumption of non-feeding, postabsorptive and inactive fish with no anaerobic activity debt (Brett & Grove 1979), and is synonymous with basal metabolism and costs of tissue maintenance. The study was carried out on juvenile Atlantic salmon, *Salmo salar*, which, like other species of freshwater fish, are known to use crevices under stones on the substrate as shelters (Cunjak 1988). Salmon compete for shelter (Kemp, Armstrong & Gilvear 2005), which suggests that they are a highly valued resource (Armstrong & Griffiths 2001). Suggested benefits of sheltering for salmon have included protection from harsh environmental conditions (Valdimarsson & Metcalfe 1998), seeking refuge from the water current (Rimmer *et al.* 1984; Pickering & Pottinger 1988; Heggenes *et al.* 1993), avoidance of displacement by floods or ice (Hartmann 1965; Heggenes *et al.* 1993; Whalen, Parrish & Mather 1999) and/or sheltering from the light at cold temperatures (Cunjak 1988; Contor & Griffith 1995). However, direct avoidance of predation is now thought to be the most likely primary function of this sheltering behaviour (Valdimarsson & Metcalfe 1998; Greenwood & Metcalfe 1998). We now suggest that the use of these shelters could have further benefits if they allow a reduction in metabolic costs.

We test this idea by measuring the standard metabolic rate of salmon parr in relation to whether or not they have access to a refuge. We also assess body coloration, since this is thought to be associated with levels of physiological stress in other contexts (Abbott, Dunbrack & Orr 1985; O'Connor, Metcalfe & Taylor 1999; Höglund, Balm & Winberg 2002; Suter & Huntingford 2002).

Materials and methods

Atlantic salmon parr in their first year posthatching were caught in mid-January 2005 from the River Almond (Perthshire, Scotland) and transferred to Glasgow University. Prior to the experiment being carried out, the fish were held in circular tanks (1 m²) at 8·5 °C in aerated, re-circulated, copper-free water

under an ambient photoperiod. They were fed to satiation on dry pelleted food once a day. While in the holding tank, the fish had access to shelters in the form of large stones and lengths of semicircular piping (approximately 120 mm in diameter). They were allowed to settle in the holding tank for 2 months before the first respirometry experiments took place. During the first month the water temperature was increased to 10 °C, but was then kept constant (±0·5 °C) throughout the remaining settling period to allow acclimation. This increase in water temperature was carried out in order to reflect the mean ambient temperature found in their natal river over the course of the next month. In order to measure standard metabolic rates (SMR), two fish were selected each day and placed without food in individual respirometer chambers to settle and evacuate their gut contents overnight (a minimum of 21 h in chamber and 48 h from last feeding before the first measurements were taken); this time period is sufficient to allow their metabolic rate to drop to resting levels (McCarthy 2000). The same sized chambers were used for each fish tested to control for any effect of chamber size on metabolic rate. The respirometry chambers (150 mm by 100 mm by 100 mm and made of clear Perspex) were placed inside two aerated water baths and each received a continual pumped input of aerated water. One chamber contained a shelter, while the other one had no internal structures. The shelter (placed in the centre of the chamber) was made from clear, semicircular Perspex (radius 20 mm, 120 mm in length) so that light from the overhead illumination remained relatively similar between the two treatments. Light intensity within the chambers was measured using a Skye Instrument SKL 300 display meter (Skye Instruments Ltd, Llandrindad Wells, Powys) connected to a SKL 310 lux sensor and was recorded as 5·50 lux, dropping to 4·31 lux under the shelter. The apparatus was screened with black plastic sheeting along the front and sides to minimize disturbance. The temperature of the room where the respirometry measurements took place was recorded as 10 °C (±0·5) throughout the entire experimental period.

SMR was measured as the reduction in oxygen concentration over time, using intermittent flow (or open-closed) respirometry. This method is described in greater detail by Steffensen, Johansen & Bushnell (1984) and Herskin (1999), and so only summary details are given here. Oxygen concentrations in the water exiting from the respirometry chamber were measured using a Strathkelvin instrument S1130 oxygen electrode (Strathkelvin Instruments Ltd, Glasgow, Scotland) contained within an SI EH100 electrode holder (Strathkelvin Instruments Ltd, Glasgow, Scotland), linked to a SI 928 6-channel meter (Strathkelvin Instruments Ltd, Glasgow, Scotland). To make a measurement of oxygen consumption, the oxygen content of the water was measured initially (t_0) when the system was in the open position (i.e. the chamber was receiving a continual pumped supply of aerated water from the water bath). Then the

system was closed for 30 min by means of valves so that the water within the chamber was continually recirculated around a closed loop (volume including pump, chamber and tubes was ≈ 1·6 l). At the end of the period of closure (t_1) the valves were returned to their original positions, so allowing fully aerated water to again be pumped through the chamber. The oxygen concentration was recorded continually from 10 min before the system was closed until 10 min after it was re-opened. A plastic screen was placed between the chamber and valves so that the valves could be adjusted out of sight of the fish; moreover, the arrangement of pumps and valves was such that the rate of flow of water through the chamber did not alter noticeably when the system was switched from the open to the closed position. At no point did oxygen concentrations drop below 90% saturation.

Recordings of oxygen consumption for each fish were first taken at 11.00 h, 21 h after being put into the chamber, and then again at 12.30 h. After the second recording the fish were removed, weighed and then placed into a separate holding tank. The water bath was drained, refilled with fresh water and the electrodes recalibrated using a solution containing an oxygen saturation of zero (a pinch of sodium sulphite anhydrous dissolved in a small amount of water). Two new fish were then put into the chambers and the entire procedure was repeated until 14 pairs of fish had provided suitable data (i.e. both fish were inactive throughout the period of measurement – see below). The shelter was switched between chambers after the seventh pair to control for any possible effects of chamber location. Oxygen consumption by the fish was calculated from the rate of decline of oxygen in the closed respirometer. The two measurements of metabolic rate on each fish were very consistent (repeatability = 0·77, Lessells & Boag 1987), and so the mean of the two SMR values for each fish was used for subsequent analysis. The values for metabolic rate for each pair of fish tested on a given day were then compared using a paired *t*-test.

Continuous behavioural observations were taken by watching the fish during the entire closed period of 30 min; the fish's activity, position in the chamber and colour were noted. As this experiment was carried out to measure standard metabolic rate, only fish resting on the bottom of the chamber during the experimental period were used; these were the majority as, after the initial settling period, fish spent virtually all their time resting motionless on the bottom of the respirometry chamber (since the water current was not strong enough to induce swimming against the flow). Any fish that did not settle during the experimental period ($n = 5$) were removed and replaced by another fish. The fish within the other chamber was also removed and replaced by another fish to prevent any acclimation differences between the two treatments. The position of the resting fish in the chamber (either with or without a shelter) was also recorded. Fish positioned in the centre of the chamber (either in or just outside

the shelter, if provided) were given a score of 0 while those that remained around the edges of the chamber received a score of 1. The overall score of the position of fish (at the edge or centre of chamber) from each treatment group was compared using a χ^2 test.

Juvenile salmon normally have a cryptic pattern of coloration on their back and sides, and are capable of adjusting their skin coloration to some degree to blend in with their surroundings. However, skin and eye (sclera) patterning can also darken rapidly, notably in losers of aggressive encounters (O'Connor *et al.* 1999). In order to ascertain whether dorsal and lateral skin and eye pigmentation varied depending on shelter presence, each pair of fish (one with and one without a shelter) was compared during the two periods the system was closed. The lighter coloured fish of the pair was given a score of 0 while the darker was scored 1. A colour difference was clearly evident in all tested pairs of fish, and was consistent between the two periods of measurement. The relative frequency with which the fish with a shelter was darker or lighter than its corresponding fish without a shelter was compared using a binomial test.

Results

The weight of the fish without shelter ranged from 3·19 to 7·76 g (mean weight 4·36 g ± 1·51 SD) while the weight of fish with shelter ranged from 3·01 to 6·93 g (mean 4·68 g ± 1·16). There was no difference between the weight of fish used in the two treatment groups (paired *t*-test, $t = 0·70$, 13 df, $P = 0·496$). The fork length of each fish was also measured and ranged from 66 to 90 mm (mean length 72·71 mm ± 7·65) in the fish without shelter and 66–84 mm (mean 76·29 mm ± 6·97) in those with shelter. Again there was no significant difference between the length of fish used in the two treatment groups (paired *t*-test, $t = 1·30$, 13 df, $P = 0·216$).

Given the lack of size difference between the treatment groups and the small range of body sizes used in the experiment, metabolic rate was expressed as oxygen consumption per unit body weight per hour. Fish with shelter had a mean SMR of 197·44 mg $O_2 \, kg^{-1} \, h^{-1} \pm 39·34$ SD (first measurement) and 191·62 mg $O_2 \, kg^{-1} \, h^{-1} \pm 46·89$ (second measurement). Fish without access to shelter had a mean SMR of 264·78 mg $O_2 \, kg^{-1} \, h^{-1} \pm 81·19$ SD (first measurement) and 262·30 mg $O_2 \, kg^{-1} \, h^{-1} \pm 86·54$ (second measurement). Comparison of the two treatments showed that the presence of a shelter led to a significant reduction in the mean resting metabolic rates of the fish (paired *t*-test comparing fish with/without shelter on a given testing day: $t = 3·52$, 13 df, $P = 0·004$; Fig. 1).

Behavioural observations revealed that the fish with a shelter appeared to rest inside it only when they were first put into the chamber. When observed the following morning they were usually lying on the bottom of the chamber against the outside edge of the shelter. Comparison of the two treatments in relation to their
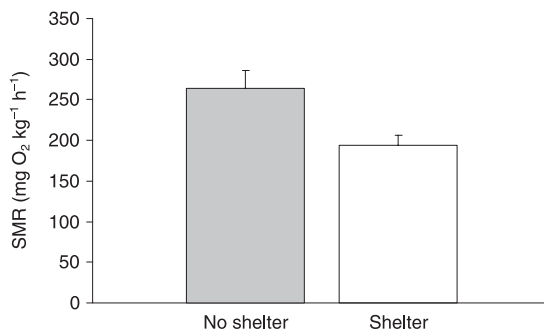
Fig. 1. Comparison of standard metabolic rate (SMR) of Atlantic Salmon parr in the absence and presence of a single shelter. The data are presented as means + SEM. The difference between the two groups was significant (paired t-test: $t = 3.52$, 13 df, $P = 0.004$).

position, categorized as the number of fish positioned at the edge *vs* the centre, showed that fish with a shelter preferred resting in the centre of the chamber near to the shelter while those without a shelter preferred to wedge themselves at the edge of the chamber. In total, 86% of the fish with a shelter preferred to rest at the centre of the chamber compared with only 43% of the fish without a shelter (χ^2 test, $P = 0.046$). Moreover, the fish that had access to the shelter was lighter in both skin and sclera coloration than the corresponding fish without a shelter in 13 of the 14 pairs, which is significantly more frequent than expected from a random association (binomial test, $P = 0.0017$). The fish with the lighter coloration also had the lower metabolic rate in all of the 14 pairs of fish used.

Discussion

The fish without access to a shelter had the higher standard metabolic rate in all but one of the 14 pairs of fish. The magnitude of this difference was substantial, averaging a 30% increase in metabolic costs in the absence of shelter. In all cases the fish in a test pair (shelter/no shelter) with the higher metabolic rate also had the darker coloration. However, while the salmon hid under the shelter when first introduced into the respirometry chamber, once they had settled (and during readings of metabolic rate) they rested immediately adjacent to the shelter rather than inside it. Therefore, the reduction in metabolic rate appears to be associated with the fish being aware of the availability of a nearby shelter rather than their current use of that shelter.

There was no suggestion that the between-treatment variation in metabolic rate was due to differences in activity, since in all 14 pairs the salmon were resting immobile on the floor of the chamber and were experiencing a water flow that was too low to require active resistance. There are several factors that might explain, at least in part, the metabolic costs of shelter absence. First, it is well established that ventilation rate is elevated in Atlantic salmon fry when exposed to the immediate threat of predation, even in the absence of

the 'flight' response (Hawkins, Armstrong & Magurran 2004). This response was suggested to show a preparation for possible subsequent activity, and this indicates that predation threat can be metabolically costly even in the absence of activity, particularly when 25% of the total metabolic rate of a fish consists of gill ventilation (Hughes & Shelton 1962). This 'non-lethal' effect of predation (reviewed by Lima & Dill 1990) occurs when predators alter the physiology or behaviour of the prey in such a way as to reduce growth rates, increase energetic demand and ultimately decrease fitness (Cooke *et al.* 2003). Increase in outputs of the opercular and cardiac pumps may result from release of vagal tone and changes in the adrenergic function associated with stress (Mazeaud & Mazeaud 1981). This adrenergic response results in the catecholamine hormones adrenaline and noradrenaline being released into the circulation from the chromaffin cells when exposed to a wide range of internal and environmental stressors (Reid, Bernier & Perry 1998). One of the primary roles of plasma catecholamines is to modulate cardiovascular and respiratory function in order to maintain adequate levels of oxygen in the blood and therefore a sufficient supply to the tissues (Reid *et al.* 1998). It is possible that those fish with shelter nearby can reduce their resting metabolism because a short anaerobic burst is sufficient to reach cover should it become necessary to flee from a predator. However, in the absence of shelter, escape may require prolonged swimming activity and fish may have to be in a sustained state of physiological readiness for such activity. This would entail a greater sustained metabolic investment in fuelling cardiac and respiratory pumps and possibly other associated changes, such as osmoregulation.

A second factor that may influence metabolism is the state of mental alertness. It is well established that vigilance can have time costs in the sense of lost feeding opportunities (Carrascal & Moreno 1992; Fritz, Guillemain & Durant 2002; Fortin *et al.* 2004). However, mental activity is metabolically demanding and so heightened vigilance may increase energy costs with various knock-on consequences (Roulin 2001). Persons *et al.* (2002) reported that antipredator behaviour of the Wolf Spider, *Pardosa milvina*, increased energy costs and decreased foraging efficiency, which resulted in both fewer and lighter egg sacs being produced compared with spiders with reduced predator vigilance. In other studies, the ability to process information effectively has been shown to reduce during long periods of vigilance (Nuechterlein, Parasuraman & Jiang 1983; Warm 1984; Parasuraman & Mouloua 1987). The nervous and immune systems are tightly linked (Maier, Watkins & Fleshner 1994) and so a requirement for high-level vigilance may also trade off against the efficiency of the immune system (Roulin 2001). Therefore the use of refuges (especially during periods when predation risk is high) may reduce the physiological costs associated with increased vigilance, so providing indirect benefits as well as the more evident protection

from predators. However, an additional cost may be incurred by the fundamental demands of maintaining sensory apparatus at a sustained elevated state of acuity. Such may be the case for those fish without shelters for which early detection would be most important.

This study showed that 13 out of 14 fish without a shelter were significantly darker than the corresponding fish with a shelter. Changes in skin coloration have been associated with the signalling of social status in salmonids, since social subordination results in rapid darkening of the skin and sclera (Abbott *et al*. 1985; O'Connor *et al*. 1999; Höglund, Balm & Winberg 2000) and communication by means of rapid, reversible visual signals mediated through differential colour patterns plays an important role in the control of aggressive behaviour in fish (Huntingford & Turner 1987). However, in the present experiment all fish were in individual chambers and had no contact with others, and so changes in coloration are unlikely to be linked to social signalling. Moreover, on each occasion the darker fish had the higher metabolic rate, suggesting that these two factors are linked in some way. While it is theoretically possible that being dark (for whatever reason) is energetically expensive, it is more plausible to suggest that the darker coloration and elevation in physiological metabolic state are linked via the stress response since skin darkening in fish appears to be related to stress (Höglund *et al*. 2000, 2002). Stress responses in teleosts are mediated via the hypothalamic–pituitary–interrenal (HPI) axis, which regulates the level of cortisol production (Donaldson 1981). It has recently been shown that the degree to which fish are conspicuous against their background can affect cortisol responses (Rotllant *et al*. 2003). Therefore, prolonged elevation in metabolic rate in the absence of shelter (and possibly because of a perceived greater vulnerability to predation) may be sustained by cortisol, which in turn leads to darkening of the skin. Such darkening may have a role in camouflage under natural conditions.

It has been shown that juvenile Atlantic salmon prefer to use refuges that allow them to hide (i.e. are dark and opaque) rather than those that shield them from the water current (Valdimarsson & Metcalfe 1998). Simulated ice cover (darkness) was shown to reduce the resting metabolism of juvenile Atlantic salmon by an average of 30% compared with fish kept without ice cover (6 h light per day) (Finstad *et al.* 2004a). While this might appear to be a similar effect to the one we report here, there are in fact several factors that could contribute to the lower metabolism of fish in darkness. Wendelaar Bonga (1993) showed that there is an increase in metabolism caused by the effect of light on melatonin secretion mediated by the pineal gland. This alone may result in the higher resting metabolic rate in fish without simulated ice cover. Alternatively, there may have been differences in activity levels between the fish held at different light levels, since Finstad *et al*. (2004a) did not monitor activity. Moreover, while the

removal of ice cover may increase the risk of predation particularly by endothermic animals, the present study highlights the fact that the availability of shelter affects metabolism even when both light and activity levels remain unchanged.

An increase in resting metabolic rate in the absence of shelter may have significant implications for growth rate, especially since a state of heightened antipredator vigilance can also result in lost foraging opportunities (Metcalfe, Huntingford & Thorpe 1987). Metcalfe *et al*. (1987) showed that increased antipredator vigilance resulted in recognition errors of food items and hence a decrease in energy intake. It has been suggested that the depletion of energy stores is the major source of winter mortality in juvenile temperate freshwater fishes, including Atlantic salmon (Gardiner & Geddes 1980; Post & Evans 1989; Miranda & Hubbard 1994; Finstad *et al*. 2004b; Biro *et al*. 2004). Fish with greater energy loss rates would be more susceptible to mortality than those with lower energy loss rates, especially during periods when feeding is energetically less profitable. Simpkins *et al*. (2003) demonstrated that an increase in metabolic demands associated with swimming activity resulted in a 20% reduction in lipid mass and a 40% reduction in triacylglyceride concentrations, compared with those of sedentary fish. However, an increased metabolism owing to the lack of appropriate refuges may also indirectly influence winter survival since this would ultimately increase the rate at which energy is lost. Therefore, if this heightened rate of metabolism was maintained over long periods, it could result in poorer growth or survival of low-energy fish in habitats where refuges are scarce, and might help explain the higher overwinter survival of trout in sites with shelters compared to sites without, even when predators were excluded (Smith & Griffith 1994). It also provides another reason for the extent to which fish will compete aggressively for shelters when these are limiting (Gregory & Griffith 1996; Harwood *et al*. 2002). More generally, the study shows that resting metabolism may vary, independently of activity levels or nutritional state, simply because of changes in the physical structure of the external environment.

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