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BEHAVIORAL PLASTICITY IN RAINBOW TROUT (*Oncorhynchus mykiss*) WITH DIVERGENT COPING STYLES: WHEN DOVES BECOME HAWKS

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ABSTRACT

Consistent and heritable individual differences in reaction to challenges, often referred to as stress coping styles, have been extensively documented in vertebrates. In fish, selection for divergent post-stress plasma cortisol levels in rainbow trout (O*ncorhynchus mykiss*) has yielded a low (LR) and a high responsive (HR) strain. A suite of behavioural traits is associated with this physiological difference, with LR (proactive) fish feeding more rapidly after transfer to a new environment and being socially dominant over HR (reactive) fish. Following transport from the UK to Norway, a switch in behavioural profile occurred in trout from the 3rd generation; HR fish regained feeding sooner than LR fish in a novel environment and became dominant in size matched HR-LR pairs. One year after transport, HR fish still fed sooner, but no difference in social dominance was found. Among offspring of transported fish, no differences in feeding were observed, but as in pre-transported 3rd generation fish, HR fish lost fights for social dominance against size matched LR opponents. Transported fish and their offspring retained their distinctive physiological profile throughout the study; HR fish showed consistently higher post-stress cortisol levels at all sampling points. Altered risk taking and social dominance immediately after transport may be explained by the fact that HR fish lost more body mass during transport than did LR fish. These data demonstrate that some behavioural components of stress coping styles can be modified by experience, whereas behavioural plasticity is limited by genetic effects determining social position early in life story.

KEYWORDS: physiology, limited plasticity, coping strategy, social dominance

INTRODUCTION

Within the last decade, it has been clearly established that individual animals of various species (vertebrate and invertebrate) differ in the nature of their response to challenges. Such differences may involve suites of correlated physiological and behavioural traits and are often referred to as "coping strategies" (Koolhaas et al., 1999). In mammals and birds, two distinct coping strategies can be distinguished. At one extreme, animals with a proactive coping strategy tend to show a fight-flight response, to be more aggressive and bold (in the sense of taking risks in a variety of dangerous situations), and to have low levels of plasma corticosteroids. In contrast, at the other extreme, reactive animals show a freeze-hide response; tend to be shy and less aggressive with a more flexible behaviour than proactive individuals. The physiological response to stress in reactive individuals involves relatively higher plasma cortisol levels, as well as differences in a number of other neuro-endocrine systems (Koolhaas et al., 1999; Korte et al., 2005).

In several species it has been shown additionally that such differences in physiological and behavioral stress responses are heritable (Van Oers et al., 2005; Øverli et al., 2005; Koolhaas et al., 2007), which raise a question about how such variability is maintained within populations. The emerging consensus is that proactive and reactive animals flourish in different selective environments, possibly in a frequency-dependent manner. Some authors have likened proactive and reactive animals to the hawks and doves of classical game theory (see for example Korte et al., 2005). The existence of such adaptive individual differences within a population has evoked considerable scientific interest and has important consequences for disciplines as diverse as evolutionary ecology (Bolnick et al., 2003; Sih et al., 2004), animal

husbandry (Cavigelli, 2005; Huntingford and Adams, 2005) and biomedicine (Korte et al., 2005).

One outstanding question is the relationship between behavioural plasticity and underlying physiological mechanisms (Øverli et al., 2007). On the one hand, if behaviour is closely linked to strongly heritable physiological traits (as in Miller et al., 2004; Uhart et al., 2004; Serretti et al., 2006; Poirier et al., 2007), this may limit behavioural plasticity. On the other hand, some components of the neuroendocrine machinery are rapidly modifiable by experience (e.g. Burmeister et al., 2005; Burmeister, 2007; Watt et al., 2007) and this is likely to be reflected in plasticity in associated behavioural traits. The results presented in this paper provide an example of flexible dissociation of behavioural and physiological components of the coping strategy, using an established fish model, the rainbow trout (*Onchorhynchus mykiss*, Walbaum, 1792).

A number of studies have documented the existence of co-varying behavioural traits in teleost fish (e.g. Bell and Sih, 2007; Wilson and McLaughlin, 2007). For example, a positive correlation between aggression towards conspecifics and risk-taking in various potentially dangerous situations has been described in three-spined sticklebacks (*Gasterosteus aculeatus*, Huntingford 1976, though see Bell and Stamps 2004), brown trout (*Salmo trutta*, Sundström et al., 2004), and grayling (*Thymallus thymallus*, Salonen and Peuhkuri, 2006). There is relatively little information on physiological correlates of such behavioural variability in fish, though Bell et al., (2007) report a correlation between individual risk-taking behaviour and brain biochemistry in sticklebacks. The rainbow trout provides an exception, in that a number of behavioural differences have been reported in strains of rainbow trout selected for high (high responsive, or HR trout) and low (low responsive, LR) cortisol

responsiveness to a standardized stressor (see reviews by Øverli et al., 2005; Schjolden and Winberg, 2007).

The first behavioural study conducted on these strains documented that fish from the LR strain become socially dominant over HR fish (Pottinger and Carrick, 2001). In addition, following transfer from group rearing to isolation in an unfamiliar tank, trout from the LR strain resumed feeding earlier than did fish from the HR strain (Øverli et al., 2002a). It was later shown that rapid resumption of feeding following transfer to a novel environment also predicts social dominance and level of aggression towards territorial intruders in non-selected aquaculture strains of rainbow trout (Øverli et al., 2004; Schjolden et al., 2005a). Most of the behavioural characteristics of the HR line are consistent with previously reported effects of the steroid hormone cortisol in non-mammalian vertebrates (Gregory and Wood, 1999; Øverli et al., 2002b; DiBattista et al., 2005). However, it seems unlikely that cortisol alone is responsible for controlling all behavioural aspects of stress coping style (Koolhaas et al., 2007; Øverli et al., 2007).

In this paper we present data indicating that cortisol responsiveness and behavioural profiles may be uncoupled. After 3 generations showing distinctive behavioural profiles, a batch of HR and LR rainbow trout were transported from their original rearing site (Windermere, UK) to Oslo, Norway. Unexpectedly, immediately after transport both strains switched behavioural profiles, with HR fish now being bolder in terms of rapid resumption of feeding behaviour after transfer to isolation and exhibiting social dominance over LR fish. The divergence in post-stress plasma cortisol concentrations between strains remained unchanged in transported as well as non-transported fish from the same generation. These data suggest a degree of

plasticity in the behavioural aspects of coping style in fish that is independent of hypothalamus-pituitary-interrenal function.

MATERIALS AND METHODS

Outline of screening regime

Results presented here come from studies carried out on the third and fourth generations of LR and HR rainbow trout. Observations were made both at the original rearing site (CEH Windermere, UK) and after transport between this site and an experimental facility in Norway. Weights below are given as mean ± S.E.M. Details of the selection programme used to generate the HR and LR strains have been described previously (Pottinger and Carrick, 1999 and 2001), as has their typical behaviour (Pottinger and Carrick, 2001; Øverli et al., 2002a; Schjolden et al., 2005a; Schjolden and Winberg, 2007).

In the summer of 2005, adult 3rd generation HR (n = 150, weight 493 ± 12 g) and LR fish (n = 150, weight 477 ± 9 g) were fitted with Passive Integrated Transponder (PIT) tags and transported from the Windermere Laboratory of the UK Centre for Ecology and Hydrology (Windermere, UK) to the Norwegian Institute of Water Research Marine Research Station (Solbergstrand, Akershus County, Norway). Fish were deprived of food for 5 days prior to transport and then loaded in a tank truck equipped to transport fish (Donslund Special Transport, Hejnsvig, Denmark). Transported fish arrived two days later and no mortalities occurred during transport. Upon arrival in Norway all PIT-tags were registered, fish were weighed and screening for behavioural and physiological traits initiated. Individual identification of fish with PIT-tags permitted body mass loss during the 7 day period of transport to be assessed, since energetic status is a variable that may strongly influence risk-taking and social

behaviour (e.g Johnsson et al., 1996; Damsgård and Dill, 1998); however, due to logistic reasons the body mass of some fish could not be measured.

Four sessions of behavioural testing were carried out. Immediately after transport adult HR (n = 23 weight: 448 ± 21 g) and LR fish (n = 23 weight: 457 ± 16 g) were tested for risk-taking (boldness). Data for this trait are not available for non-transported fish, but previous studies reported that adult LR fish resume feeding more rapidly than HR fish at the original rearing site (Øverli et al., 2002a). Social dominance and plasma cortisol following an acute stressor were also assayed in Norway. Simultaneously in Windermere UK, non-transported 3rd generation adult fish (n = 16 weight: HR 742 ± 41 g, n = 16; LR 645 ± 28 g) were tested for social dominance and post-stress plasma cortisol. In Norway, one year later in the summer 2006 a set of HR (n = 18 weight: 1113 ± 65 g) and LR (n = 18 weight: 1108 ± 61 g) fish, transported the year before, were screened for the same behavioural traits as in 2005. Finally, 8 months old 4th generation offspring (n = 16 weight, HR 16.2 ± 0.9 g, n = 16; LR 16.4 ± 0.9 g,) generated from transported F3 fish were screened for the same behavioural and physiological traits in August 2006.

Screening for boldness and social dominance

Prior to screening, fish were transferred from communal rearing tanks to 250 l (adult fish) or 12 l (offspring) glass aquaria, where they were held in isolation. Each observation aquarium was lined with black plastic on three sides and divided in two compartments by a removable opaque PVC wall, each section holding one HR or one LR fish in weight matched pairs. Prior to transfer from group rearing to social isolation each fish was anaesthetised, weighed, and for adult fish the PIT-tag was read. Offspring were fin clipped to distinguish between strains, by a small incision in the upper or lower section of the tail fin.

Fish were allowed to recover overnight and testing started the next day. For seven consecutive days, feeding was performed by dropping appropriate sized pellets where the fish was able to perceive them. Feeding was stopped either when the fish had refused to eat three pellets in a row or had consumed 3.0% of its body mass. Uneaten food was removed immediately after. Feeding behaviour was graded on each occasion according to the criteria listed in table 1, in accordance with the method presented by Øverli et al. (2006). There is a high degree of individual variation in the speed of progress from low to high scores on this scale. Accumulated scores during the course of an experiment thus reflect how quickly fish resume normal feeding, and have previously been used as a measurement of risk-taking or boldness in rainbow trout (see Øverli et al., 2006, 2007).

After the 7th day of feeding, the dividing screen in each aquarium was removed and fish were allowed to interact until the conflict was resolved and dominant and subordinate fish could be clearly distinguished. For all the experimental sites the conflict did not last more than 3 hours, fish were removed from the experimental tanks immediately after to avoid unnecessary distress. After initial fights, subordinate fish showed little or no aggression towards the dominant individual and usually took up a position facing a corner of the tank, while dominant fish normally moved around the whole territory frequently chasing the subordinate fish (Øverli et al., 1999; Pottinger and Carrick, 2001). After a stable dominance-subordination relationship was confirmed, fish were netted, anesthetized, and the final weight and strain of each winner and loser was recorded. A similar procedure was also carried out on F4 offspring in summer 2006. These fish were transferred from group rearing in 150 l holding tanks to feeding and dominance tests in 12 l

observation aquaria (HR: n = 15, LR: n = 15), or subjected to an acute stress test (see below).

The above procedures applied to tests carried out in Norway. In 2005 the Windermere laboratory was not equipped to carry out feeding tests in glass aquaria, so at this location adult F3 fish were assessed for the ability to gain dominance over a size-matched competitor, using 50 l PVC tanks. Size-matched fish from the two lines (16 pairs) were held in 50 l tanks for seven days by which time all fish were feeding normally. They were then paired (HR, LR) by transfer to a new 50 l tank and the positions of the fish were noted after 3h of interaction. Within each pair the identity of each fish was denoted by a panjetted alcian blue dye mark. The latter test was carried out to exclude the possibility that the ability to gain social dominance in HR fish is an age-dependent phenomenon, as previous studies were carried out on juvenile fish (Pottinger and Carrick, 2001).

Stress testing and hormone assays

For analysis of the plasma cortisol levels for transported fish, in July 2005 adults of the 3rd generation (HR n = 20, LR n = 15) were subjected to a confinement stress test (confinement in 12.5 l of water for 0.5 h) after 7 days of rearing in isolation. Upon sampling fish were anesthetized in 0.5 g / l MS-222, and a blood sample was collected from the caudal vasculature. At CEH Windermere, in October 2005, fifteen fish of each line were confined for 2 h in groups of three in a 25 l volume in polyproylene tanks receiving a constant flow of lake water. The fish were sedated in 2-phenoxyethanol (1:2000) and a 1.0 ml blood sample was collected from the Cuverian duct. Stress testing of offspring by individual confinement for 30min in 0.45 l of running water (HR n = 10, LR n = 10) was carried out in August 2006. After separation of plasma, cortisol levels were quantified using a previously validated

radioimmunoassay (Pottinger and Carrick, 2001) or (for fish sampled in Norway) a commercial enzyme linked immunosorbent assay kit (Neogen Corporation, Lexington, USA).

Statistical analysis

For the fish transported to Norway, strain differences in feeding scores as well as body mass loss were assessed with two-sample t-tests. Differences in social dominance were analysed by a sign test, calculating the likelihood that observed frequencies of dominant and subordinate fish in each strain represent a 1:1 ratio. Plasma cortisol concentrations in LR and HR fish were compared using a two-sample t-test for each sampling point.

All the procedures were carried out in accordance with the welfare regulations for each country and experimental site; in general, unnecessary distress was avoided.

RESULTS

Risk-taking

As shown in figure 1, HR fish gained higher feeding scores than LR fish immediately after transport, (t = 2.59, p = 0.013) and one year later (t = 4.24, p = 0.001). In 4th generation offspring of transported fish, LR and HR fish did not differ with respect to this trait (t = 0.50, p = 0.62).

Social dominance

As expected from previous reports (Pottinger and Carrick, 2001), see figure 2; in Windermere LR fish typically became dominant over HR fish (LR dominant in 12 of 16 pairs, p = 0.03). Immediately after transport, HR fish tended to dominate over LR fish (LR dominant in 4 of 19 pairs, p = 0.001). The following year, there was no significant difference in the proportion of transported LR and HR becoming dominant

(LR dominant in 5 of 11 pairs, p = 1.00). In offspring of transported fish the original pattern was reinstated, with LR fish becoming dominant in all pairwise encounters (15 of 15 pairs, p < 0.001).

Body mass loss in transported fish

Among adult fish tested in Norway, on average, HR fish lost almost twice as much body mass due to stress and food deprivation in connection with transport than did LR fish. (t = 3.43 p = 0.003). Mean body mass of the HR group fell from 458.38 ± 27.91 g before transport to 403.57 ± 23.33 g after transport to Norway. The equivalent figures for LR fish were 426.39 ± 18.74 g and 401.37 ± 19.34 g respectively. Body mass loss was also less severe among those few HR fish that lost pairwise contests (n = 4) than among HR winners (n = 13; t = 4.17, p = 0.014). This pattern was not evident in the LR strain, as LR winners and losers did not differ in body mass loss (n = 19; t = 0.74 p > 0.05).

Post-stress plasma cortisol concentrations

Table 2 shows post-stress plasma cortisol concentrations (mean ± S.E.M.) in transported and non-transported adult LR and HR fish, and in 4th generation offspring. There was a highly significant difference in post-stress plasma cortisol in non-transported fish, with LR fish showing significantly lower levels (t= 3.52; p = 0.003). Transported fish maintained this typical divergence in post-stress plasma cortisol (t = 3.74; p = 0.001). The offspring of transported fish also showed strain distinctive post-stress plasma cortisol concentrations (c.f. table 2, t = 2.34; p = 0.03) as well.

DISCUSSION

These results demonstrate a hitherto undisclosed level of behavioural flexibility in selected lines of rainbow trout that serve as a comparative model for the study of coping strategies. Previous studies on these lines and on non-selected strains of rainbow trout have revealed distinct behavioural profiles consistent not only over time, but also across different contexts, similar to the results of studies on proactive and reactive mammals (Øverli et al., 2005; Schjolden et al., 2005a and b; Schjolden and Winberg, 2007). These physiological and behavioural characteristics of low stress responsive (LR) and high stress responsive (HR) rainbow trout have been conserved over at least three generations (Pottinger and Carrick 2001; Øverli et al., 2005).

An unexpected change in behaviour occurred in the HR and LR strains following a prolonged stressful experience (transfer of the fish from the UK to Norway). Previously, LR-trout trout have typically been characterized as taking greater risks when feeding in a novel environment (equates to boldness, but see Øverli et al., 2007) and becoming dominant in competitive pairwise interactions with size matched HR-trout (Pottinger and Carrick, 2001, Øverli et al., 2002a and 2005, Schjolden et al., 2005a). This set of behavioural differences was present in adult fish of the third generation kept at the original rearing site. However, these traits were reversed immediately after transport to Norway, in which fish experienced a 7 day period of starvation. After transport HR fish not only resumed feeding sooner after transfer to the novel environment, but also won more pairwise fights than did LR fish.

A clue as to a possible mechanistic explanation for the switch in traits may be found in the observation that HR fish lost a greater proportion of their body mass during the transport period than did LR fish. Furthermore, the few HR fish that became subordinate after transport were characterised by having lost relatively little body mass during transport. It is well established that individuals become bolder and

more aggressive in competition for resources when their need for such resources is high (Dugatkin and Ohlsen 1990; Morrell et al., 2005; Frost et al., 2007). Thus the atypical behaviour of HR fish immediately after transport could be due to experiencing a high degree of hunger. Comparable results to those reported here were described by Carere et al. (2005), who found that food deprivation modulates the expression of phenotypic traits (namely begging and aggression) in lines of great tit (*Parus major*) selected for high and low exploration speed and aggression.

By one year after transport when all the fish had recovered from transport stress, inversion of the established behavioural profiles of the two lines was still evident. HR fish still took greater risks to feed in a novel environment, although individuals of the two strains were equally likely to win pairwise contests. It is possible that a long-term effect associated with the body mass loss that occurred during transport continued to influence the behaviour of the HR fish in pairwise fights. Growth rates between 2005 and 2006 (unpublished data) were similar in the two strains but perhaps HR fish retained a metabolic "debt" that they were unable to discharge in their group holding tanks. In the 4th generation of the two lines no differences between lines were evident in risk-taking during feeding, but LR fish now won all the paired contests. It seems that the ability to win pairwise fights is an inherited trait in rainbow trout. The situation with respect to risk-taking is more complicated and harder to explain; although there is a tendency for LR fish to feed more and sooner than HR fish in generation 4, this difference is not significant.

These changes in behaviour occurred without any associated change in a key physiological component of the coping styles, divergent post-stress plasma cortisol levels, which were maintained throughout the study period. This suggests a degree of plasticity in the behavioural aspects of coping style in fish that is independent of

hypothalamus-pituitary-interrenal function. A range of other elements of the neuroendocrine system (hormones, neuropeptides and transmitters) are also involved in the regulation of these behaviours (Winberg and Nilsson 1993; Johnsson and Björnsson, 1994; Johansson et al., 2005; Clements et al., 2003; Volkoff, 2006; Carpenter et al., 2007) but further work will be needed to assess their role in the reversal of behavioural traits observed in the present study. In conclusion, data presented in this paper suggest that in rainbow trout genetic differences determine social position only in early life. Superimposed on this template, some behavioural components of stress coping style, including resource holding potential and risk-taking, can subsequently be modified by experience.

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Ruiz-Gomez et al., table 1

Table 1. Point scores to grade feeding behaviour in fish after transfer to social isolation (reprinted from Physiology and Behaviour).

Points	Behaviour
0	Fish does not respond to food
1	Fish eats only pellets that falls directly in front, and does not move to take food
2	Fish moves more than one body length to take food, but returns to original position in aquarium between each food item
3	Fish moves continuously between food items and consumes all food presented

Ruiz-Gomez et al., table 2

Table 2. Mean and S.E.M. post-stress plasma cortisol concentrations of LR and HR rainbow trout, p-value from the two-sample test

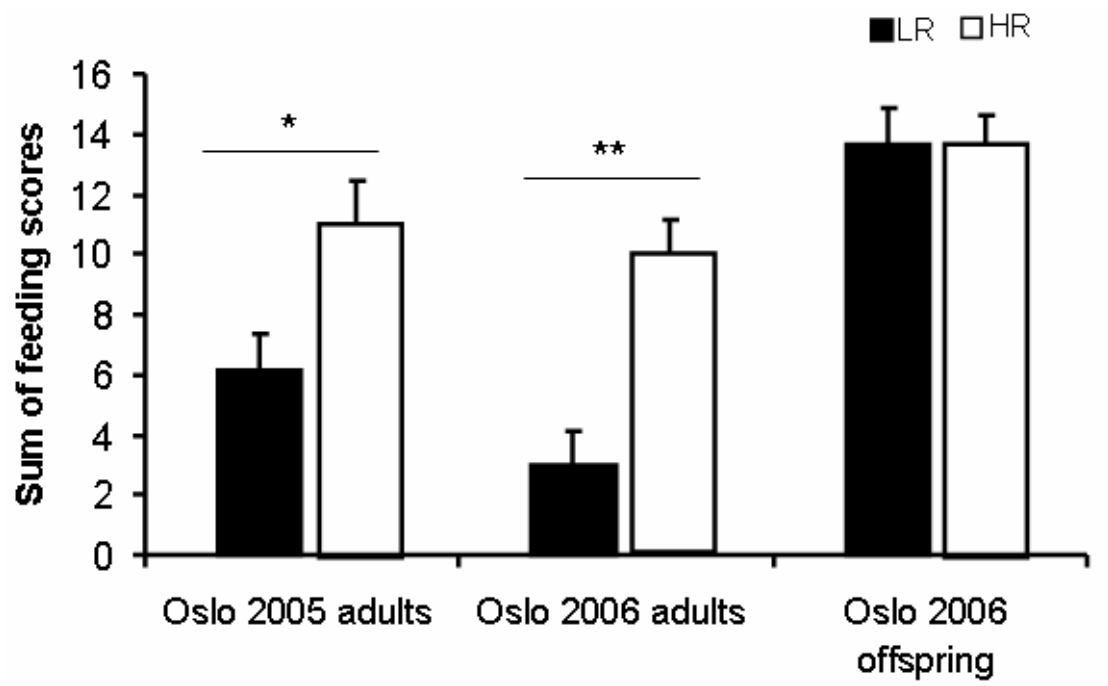|	Plasma cortisol (ng/ml)		p-value
	LR	HR	
Original rearing site (Windermere, UK)	58.3±9.5	153.1±25.0	0.003
Immediately after transport (Oslo, Norway 2005) adult fish	60.6±7.3	128.0±16.0	0.001
G4 Offspring, (Oslo, Norway 2006) juvenile fish	37.4±6.7	70.1±12.0	0.034

Figure 1. Sum of feeding scores by 7th day following transfer to isolation in HR and LR fish. * p<0.05; ** p<0.01 . Data not available for non transported fish.
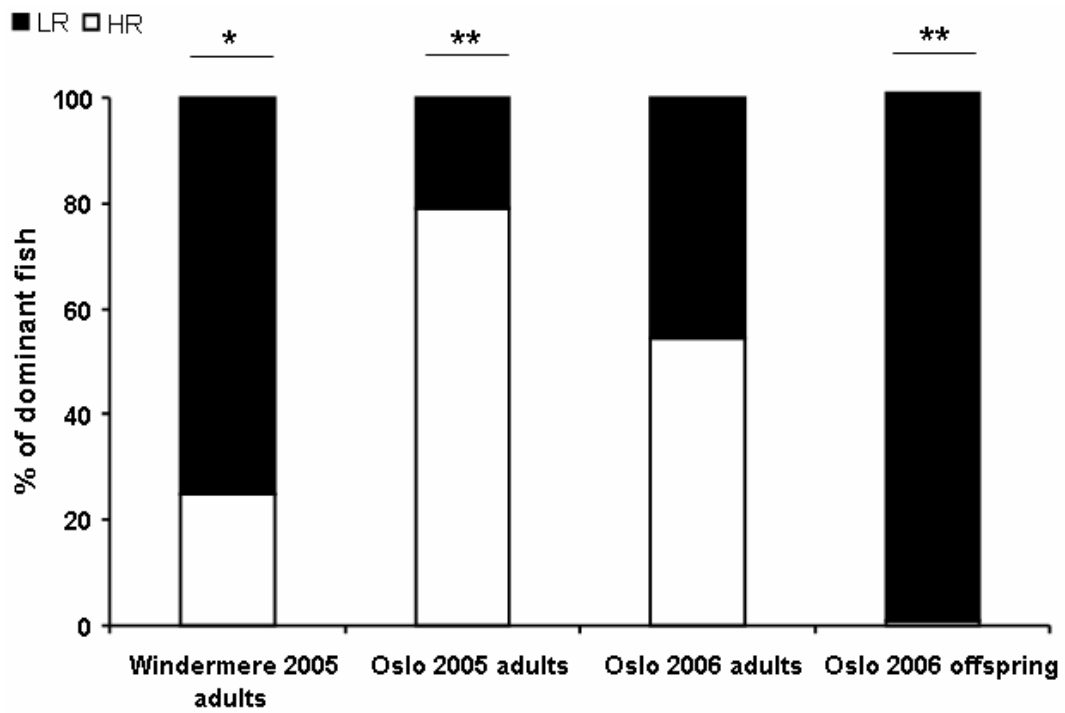
Figure 2. Percentage of LR and HR fish becoming dominant in size matched pairs on the 4 sampling points. * p<0.05, ** p<0.01