

Restless Rainbows And Bold Browns: Personality of Domesticated Trout Predicts Invasiveness And Impact

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Abstract

Personality and behavioural syndromes are increasingly recognised in many animal species and are now seen as critical to the prediction of non-native species success and impacts. Rainbow trout and brown trout have been introduced globally, but differ in patterns of establishment success and ecological impact. While environmental tolerance may be a driver, thus far the role of behaviour has not been rigorously tested. Here, we investigated whether diploid rainbow trout and diploid and triploid brown trout differ among several key behavioural measures linked to invasibility. We assessed activity, boldness, aggression, and feeding, using open field, novel object, shelter, mirror, feeding, and functional response experiments. We also tested within each fish type for behavioural syndromes comprising correlations among activity, boldness and aggression. Rainbow trout were more active and aggressive but less bold than diploid and triploid brown trout. In small groups, however, rainbow trout were bolder than both types of brown trout. Diploid brown trout were more active and bolder than triploids when tested individually. There was no association between activity and boldness in rainbow trout, but there was in both brown trout. The increased activity and aggression of rainbow trout may reflect an increased stress response to novel situations, with this response reduced in a group. These results suggest that rainbow trout do not manage their energy budgets effectively, and may explain why they are implicated in fewer ecological impacts. Comparative analyses of multiple behaviours of invasive species and genetic variants may thus be key to understanding and predicting invader success and ecological impacts.

Introduction

Personality in animals describes the tendency of individuals to have consistency in behavioural traits^[1–3]. For example, individuals which are more aggressive in one context will also be more aggressive in different contexts^[4, 5]. Many studies have also shown correlations between different types of behaviour, with these correlations termed “behavioural syndromes”^[4, 6, 7], such as the correlation between aggression and boldness in sticklebacks^[8], and live-bearing *Poecilia paras* fish exhibiting a behavioural syndrome of aggression, boldness and exploration^[9]. The evolutionary history of a species and its ecology can also lead to differences in boldness, aggression or activity between species^[4, 10]. We now appreciate that such personality and behavioural syndromes at the level of the individual may have profound impacts at the population and community levels^[11, 12].

Behaviour is increasingly seen as an important metric for explanation of the success or failure of non-native species establishment and spread^[3, 13, 14]. For example, in several species, e.g. mosquitofish and gobies, individuals at the “invasion front” are bolder, more active, and with faster metabolisms than individuals in more established populations^[15–18], suggesting that these behavioural traits promote success at different invasion stages^[19]. At the establishment stage of invasion, increased aggression and greater foraging effort may help to out-compete native analogues^[20, 21]. Additionally, shy individuals may suffer less predation than bolder individuals, and reactive (passive but more flexible in behaviour) individuals may respond more effectively to changes in the environment, promoting their

survival [22]. Differences in behaviour among native and non-native species can also affect the ecological impact of invaders, that is, changes in native populations of species due to interactions such as predation [23, 24]. For example, more aggressive and bolder individuals may have a greater impact on native analogues through direct or indirect interactions, leading to displacement and even extirpation of natives [20, 25]. The ecological impact of non-native species on potential prey can also be predicted by comparative functional response experiments, comparing invaders with native trophic analogues [26, 27]. Functional responses, which quantify *per capita* feeding rates, can also vary with invasion stage [28] and have been correlated with personalities. For example, smaller crabs with higher activity levels have a higher functional response, possibly because greater activity levels indicate more time spent actively foraging [29].

Rainbow trout and brown trout are both valuable native species and significant invasive species worldwide, with a long history of introductions in many different countries [30–32]. Both species have successfully established invading populations in Australasia [33], Japan [34], South Africa [35], the USA [36], and elsewhere. The success of rainbow trout in Europe (where brown trout is native) is, however, limited, and in the British Isles, for example, there is currently only one known self-sustaining rainbow trout population (in the River Wye, Derbyshire [37]). Where both trout species have been introduced as non-native species, brown trout appears to have a greater ecological impact on native species. For example, in New Zealand and Australia, brown trout have been implicated in many more negative effects than rainbow trout [33]. In Chilean Patagonia, however, rainbow trout were found to successfully invade a greater area than brown trout [38], although brown trout were still found to have a greater ecological impact on the native galaxiids.

One measure to ostensibly prevent the establishment of invasive trout is a triploidy treatment to prevent reproduction in the wild [39]. However, this raises the question of whether triploid individuals may have a greater or lesser ecological impact as a consequence of their different genetics and physiology [40]. Previous research has found little evidence of impact of stocked brown trout on wild analogues, with no significant difference of impact between mixed-sex diploids and all-female triploid cohorts [41], however triploid brook trout have been found to grow more slowly and are more prone to stress than their diploid counterparts, suggesting that their ability to adapt to the wild and exert impact may be reduced [42]. Triploid Atlantic salmon parr also have a reduced growth rate compared to diploids, indicating that the negative impact of the triploidy treatment may generalize across salmonids [43]. Thus, where possible, genetic variants of invasive species should be incorporated into behavioural studies.

Although trout behaviour has been studied in many different contexts previously, there have been very few direct comparisons between rainbow trout and brown trout. Lines of rainbow trout bred for different cortisol responses exhibited different behaviours under stress [44], and in brown trout there is evidence of personality [45] and a behavioural syndrome comprising activity and aggression [46]. Behavioural differences between these two species are likely to be important factors determining the more successful

invader, however this remains to be rigorously explored. The survival of both species from fry to juvenile is a critical period influencing the risk of establishment, as young fish exhaust their yolk sac and must then forage independently [47, 48], with intense competition over foraging territories necessitating aggression [48]. Therefore, in this study we compare several key behavioural traits (activity, boldness and aggression) for both species (including both ploidy variants of brown trout) at the fry stage, as well as measures of potential impact (feeding rate and functional response). We use measures of these traits to assess the evidence for behavioural syndromes in the three types of trout, and interpret our results in the ecological context of the differential success and ecological impacts of these trout as invaders, since all three types may be released or accidentally escape from aquaculture facilities.

To examine any differences in behaviour we carried out a series of previously validated tests. Activity was measured across the tests using either percentage time swimming, number of line crosses or number of times moving out from under a shelter. Boldness was also measured across several of these tests. The first of the tests was an open field test, which is commonly used to assess an individual's boldness in a novel environment [49]. The novel object test is a common test for boldness which has been used previously on diploid brown trout fry [50], and also in diploid rainbow trout [51]. We combined this test with a disturbance test to measure the effect of the object on boldness. The use of overhanging shelters in nature enables fish to evade detection by predators, thus we also carried out a shelter and simulated predation test to determine whether the species differed in boldness when offered a refuge, and their potential vulnerability to predation. To analyse differences in aggression we used a mirror test, as has been used with brown trout before [52]. Finally, a group test examined the influence of conspecific presence on boldness in each species. Moreover, we performed a fixed density feeding test to quickly assess maximal feeding rate, and then a comparative functional response experiment to quantify any differences in *per capita* feeding behavior that may explain and predict the generally lower known field ecological impacts of rainbow as compared to brown trout. By performing all of these tests, we were able to build a comprehensive picture of the behavioural dynamics of rainbow and brown trout, while also expanding our comparison to incorporate the effects of ploidy on the behaviour of brown trout.

Results

Open Field test

All results of the OFT are shown in Fig. 1.

Rainbow trout spent a greater percentage time swimming than both diploid brown trout ($t = 5.92$, $df = 66$, $p < 0.001$) and triploid brown trout ($t = 4.98$, $df = 66$, $p < 0.001$), with no difference in percentage time swimming between the two brown trout types. Rainbow trout had significantly more line crosses than both diploid ($t = 4.64$, $df = 66$, $p < 0.001$) and triploid ($t = 8.66$, $df = 66$, $p < 0.001$) brown trout, and diploid brown trout had more line crosses than triploids ($t = 4.54$, $df = 66$, $p < 0.001$).

There was no difference between rainbow trout and diploid brown trout in latency to begin swimming, but rainbows were significantly faster than triploids ($t = 4.05$, $df = 66$, $p < 0.001$) as were diploid browns ($t = 2.39$, $df = 66$, $p < 0.001$).

For the location data, rainbow trout spent significantly less time in the perimeter than triploid browns ($t = 2.50$, $df = 66$, $p < 0.05$) and diploid browns spent less time in the perimeter than triploids ($t = 2.46$, $df = 66$, $p < 0.05$). Rainbow trout also spent significantly more time in the outer ring than triploid browns ($t = 3.83$, $df = 66$, $p < 0.001$). All other comparisons were non-significant. There was no significant effect of body mass on any of the behaviour variables measured in the OFT.

Disturbance/Novel object test

All results of the disturbance/novel object test are shown in Fig. 2.

In the disturbance test, rainbow trout had significantly more line crosses than triploid brown trout ($t = 2.39$, $df = 135$, $p < 0.05$), but the remaining comparisons across fish type for number of line crosses were not significant. There was also no significant effect of fish type on latency to approach the centre in the disturbance test.

In the novel object test, rainbow trout had almost significantly fewer line crosses than diploid brown trout ($t = 2.23$, $df = 135$, $p = 0.06$) and diploid brown trout had more line crosses than triploids ($t = 3.23$, $df = 135$, $p < 0.01$). Rainbow trout were significantly slower to approach the novel object than diploid brown trout ($t = 2.97$, $df = 135$, $p < 0.01$), but there were no significant comparisons with triploid brown trout.

The type of test (disturbance or novel object) affected the three types of fish differently. There was no significant effect of the novel object on the number of line crosses for rainbow trout or triploid brown trout, but diploid brown trout had significantly decreased number of line crosses ($t = 3.4$, $df = 135$, $p < 0.001$). Rainbow trout were significantly slower to approach the central ring in the novel object than in the disturbance test ($t = 2.55$, $df = 135$, $p < 0.05$) whereas diploid brown trout were almost significantly faster to approach the central ring in the novel object test ($t = 1.86$, $df = 135$, $p = 0.06$), with no significant effect in triploids.

The three types of fish did not differ in the percentage time spent in each part of the arena, but the type of test had a significant effect. Significantly less time was spent in the perimeter ($t = 3.11$, $df = 137$, $p < 0.001$) and significantly more time in the outer ring ($t = 3.41$, $df = 137$, $p < 0.001$) in the novel object compared to the disturbance test.

Shelter/predation test

All results for the shelter and predation tests are shown in Fig. 3.

There was no significant difference between rainbow trout and diploid brown trout for number of shelter crosses, but rainbow trout had significantly more than triploid brown trout ($t = 2.62$, $df = 137$, $p < 0.05$) as did diploid brown trout ($t = 3.09$, $df = 137$, $p < 0.01$).

For both tests, rainbow trout spent significantly less time outside the shelter compared to diploid brown trout ($t = 2.04$, $df = 137$, $p < 0.05$), although no comparisons with triploid brown trout were significant.

There was no significant interaction between type of fish (rainbow trout, diploid or triploid brown trout) and type of test (whether shelter or predation). Instead, number of shelter crosses was significantly decreased by the predation effect ($t = 4.89$, $df = 137$, $p < 0.001$), and the percentage time spent outside the shelter also decreased ($t = 2.37$, $df = 137$, $p < 0.05$) compared to the shelter test for all three types of fish.

Mirror test

All results of the mirror test are shown in Fig. 4.

Rainbow trout were significantly faster to initiate aggressive swimming along the mirror than diploid brown trout ($t = 3.17$, $df = 65$, $p < 0.01$), and triploid brown trout ($t = 3.55$, $df = 65$, $p < 0.01$) with no difference between diploid and triploid brown trout. Heavier fish were also faster to initiate this aggressive behaviour ($t = 2.40$, $df = 65$, $p < 0.05$).

Rainbow trout also spent a significantly greater proportion of time in the active zone near the mirror than diploid brown trout ($t = 2.40$, $df = 65$, $p < 0.05$). Neither rainbow trout nor diploid brown trout differed significantly from triploids in proportion of time spent in the active zone.

Regarding the time spent within the active zone, fish types did not differ significantly in aggression or freeze behaviour, but triploid brown trout spent a smaller proportion of time swimming passively away from the mirror than rainbow trout ($t = 2.48$, $df = 66$, $p < 0.05$) and almost significantly more than the diploid brown trout ($t = 2.26$, $df = 66$, $p = 0.068$).

Feeding test

Feeding rate did not differ significantly by fish type or mass of fish. Only one variable was found to significantly improve the null model explaining feeding rate, the latency to initiate aggressive swimming in the mirror test, with the effect approaching significance ($t = 1.76$, $df = 64$, $p = 0.08$).

Correlational analysis

Rainbow trout:

The association between activity and boldness in rainbow trout was unclear, with both directions of correlation observed. The percentage time spent swimming in the OFT was negatively correlated with the percentage time spent outside the shelter ($r = -0.45$, $df = 22$, $p < 0.05$), however, the inverse of the latency (1/latency) to approach the novel object was positively correlated with number of shelter crosses ($r = 0.60$, $df = 22$, $p < 0.01$).

Boldness was negatively correlated with aggression, as shown by a negative correlation between percentage time spent in the inner rings in the novel object test and the percentage time in the active zone of the mirror test spent aggressively swimming ($r = -0.56$, $df = 22$, $p < 0.01$).

Activity was positively correlated with aggression along one measure, number of line crosses in the novel object test with the percentage time spent in the active zone of the mirror test ($r = 0.48$, $df = 22$, $p < 0.05$). No significant correlations were found across the behaviours with mass of the fish or number of bloodworms eaten. All correlations for rainbow trout are illustrated in Supplementary Figure S3.

Diploid brown trout:

Activity correlated with boldness in diploid brown trout, as shown by a single correlation between the 1/latency to approach the novel object and the number of shelter crosses ($r = 0.61$, $df = 22$, $p < 0.01$).

There was also a correlation between activity and aggression in diploid brown trout, with the number of shelter crosses significantly correlating with 1/latency to begin aggressive swimming ($r = 0.43$, $df = 22$, $p < 0.05$).

There were, however, no significant correlations between boldness and aggression variables, or for the number of bloodworms eaten with any of the behavioural measures. The mass of fish positively correlated with a boldness measure, percentage time spent in inner rings ($r = 0.42$, $df = 22$, $p < 0.05$), and an activity measure, number of line crosses in the novel object test ($r = 0.45$, $df = 22$, $p < 0.05$). All correlations for diploid brown trout are illustrated in Supplementary Figure S4.

Triploid brown trout:

Activity positively correlates with boldness in triploid brown trout, as shown by two measures: percentage time spent swimming in the OFT with percentage time spent outside the shelter ($r = 0.59$, $df = 22$, $p < 0.01$), and 1/latency to move in the OFT with the number of shelter crosses ($r = 0.50$, $df = 22$, $p < 0.05$).

There is a positive correlation between activity and aggression, as shown by the association between the percentage time spent swimming in the OFT with 1/latency to begin aggressive swimming ($r = 0.42$, $df = 22$, $p < 0.05$), the number of shelter crosses with 1/latency to begin aggressive swimming ($r = 0.53$, $df = 22$, $p < 0.01$) and between the percentage time spent aggressively swimming in the active zone of the mirror test with the number of shelter crosses ($r = 0.51$, $df = 22$, $p < 0.05$), the number of line crosses in the novel object test ($r = 0.44$, $df = 22$, $p < 0.05$), and the percentage time spent swimming in the OFT ($r = 0.54$, $df = 22$, $p < 0.01$). There was however, a negative correlation between the percentage time spent in the active zone in the mirror test and the number of shelter crosses ($r = -0.46$, $df = 22$, $p < 0.05$).

Boldness also correlates with aggression as shown by a positive correlation between the percentage time spent being aggressive in the active zone of the mirror test and 1/latency to move in the OFT ($r = 0.52$, $df = 22$, $p < 0.05$), and with the percentage time spent outside the shelter ($r = 0.41$, $df = 22$, $p < 0.05$).

Heavier triploid brown trout were more aggressive, as shown by a positive correlation between mass and 1/latency to begin aggressive swimming ($r = 0.49$, $df = 22$, $p < 0.05$). Fish with a higher feeding rate were also less active and less aggressive, as shown by negative correlations with the percentage time spent

swimming in the OFT ($r = -0.43$, $df = 22$, $p < 0.05$) and $1/\text{latency}$ to begin aggressive swimming ($r = -0.42$, $df = 22$, $p < 0.05$). All correlations for triploid brown trout are illustrated in Supplementary Figure S5.

Group test

The best model for the group test included fish type and mean mass, but no interaction between fish type and type of test (disruption or novel object) (Fig. 2C). Rainbow trout had a significantly faster latency of the first fish to approach the central ring than diploid brown trout ($t = 3.71$, $df = 14$, $p < 0.01$), and triploid brown trout ($t = 3.19$, $df = 14$, $p < 0.05$) (Fig. 2C), with no significant difference between diploid brown trout and triploid brown trout. There was an almost significant effect of mean mass, with heavier groups with a faster latency ($t = 1.86$, $df = 14$, $p = 0.08$).

Functional response trials

Prey survival in the predator-free controls was 100%, and therefore experimental deaths were attributed to predation. For all predators, Type II FRs were recorded, with significantly negative first-order terms in each instance (Table 2; Fig. 5). Under the novel prey treatment, triploid brown fry had the highest attack rates (triploid > diploid > rainbow), while diploid brown fry had the shortest handling times, and therefore highest maximum feeding rates (diploid > triploid > rainbow; Fig. 5a). For non-novel prey, triploid brown fry had the highest attack rates (triploid > diploid > rainbow), with diploid brown fry having the shortest handling times/highest maximum feeding rates (diploid > rainbow > triploid; Fig. 5b).

Table 2 First-order terms calculated from logistic regression to denote functional response type across all predator treatments. The significantly negative first-order term values indicate Type II functional responses. Attack rate (a), handling time (h), maximum feeding rate ($1/h$) parameter estimates were derived using Rogers' random predator equation (Eqn. 1). *** <0.001, ** <0.01. See also Figure 5.

Fish type	Novelty treatment	First-order term	Attack rate (a), p value	Handling time (h), p value	Maximum feeding rate ($1/h$, prey per 4 hours)
Rainbow diploid	Novel	-0.123 ***	13.995	0.100 ***	10.000
Brown diploid	Novel	-0.118 ***	4.720 ***	0.063 ***	15.873
Brown triploid	Novel	-0.122 ***	33.970 ***	0.092 ***	10.870
Rainbow diploid	Not novel	-0.070 ***	4.223 **	0.085 ***	11.765
Brown diploid	Not novel	-0.106 ***	5.873 **	0.045 ***	22.222
Brown triploid	Not novel	-0.116 ***	40.915 ***	0.100 ***	10.000

Discussion

We show that rainbow trout are more active, shyer and more aggressive than diploid and triploid brown trout when tested individually, but in a group rainbow trout are bolder. Behavioural syndromes also vary between the three fish types. In rainbow trout, results show a positive correlation between activity and aggression, and a negative correlation between boldness and aggression. In diploid brown trout we found a behavioural syndrome of activity with boldness and activity with aggression, however, there was no correlation between boldness and aggression, and the observed correlations were only single measures. Triploid brown trout had the strongest behavioural syndrome, with correlations between boldness, activity and aggression. The feeding rate results do not show any effect of fish type, but the comparative functional response experiments showed that diploid brown trout had the highest functional response, and that they increased their functional response to a greater degree when presented with non-novel prey. Since functional responses allow us to examine feeding behaviour in more detail we have focused on these results more than the fixed density experiment ^[27].

Rainbow trout were more active than diploid and triploid brown trout, as shown by the increased percentage of time spent swimming and more line crosses in the OFT, and the greater number of line crosses in the disruption test. This pattern was reversed during the novel object test, and was likely due to the greater boldness of the diploid brown trout in the presence of the object, since the number of line crosses is directly related to approaches into the centre of the arena, and diploid browns were faster to approach the centre. The results of these tests indicate that the relative activity of rainbow and diploid brown trout do not generalise across contexts, with brown trout being more active in the presence of novel objects, but rainbow trout more active in a new environment. Furthermore, this may reflect different motivations towards activity, with brown trout moving to take advantage of novel phenomena, and rainbow trout moving in a new environment perhaps as a consequence of a greater stress response. Rainbow trout have been shown previously to exhibit higher stress responses to the same conditions compared with brown trout ^[53]. Short term cortisol treatment increases activity and does not decrease aggression in rainbow trout ^[44], therefore the increased activity and aggression of the rainbow trout may suggest that the rainbow trout were more stressed than the brown trout during the experiment.

By being less active but more ready to respond to novel phenomena, domesticated brown trout may more effectively balance their energy requirements. This hypothesis makes sense when considering the feeding ecology of trout; both rainbow trout and brown trout fry typically capture food items as they drift past in the current. By remaining relatively still and only moving to approach novel items, brown trout may reduce their energy consumption while maximizing opportunities to feed. It has also been previously found that slower exploring brown trout grow faster since they expend less energy when foraging ^[50]. The behaviour of the rainbow trout was, however, not uniformly less adaptive than the diploid brown trout. Rainbow trout spent significantly more time under the shelter in the paired shelter/predation tests, suggesting that diploid brown trout may be more at risk of predation as a result of their greater boldness.

The presence of conspecifics had a greater impact on the behaviour of rainbow trout. For example, rainbow trout were more aggressive than both brown trout types, since they had a faster latency to initiate aggressive swimming in the mirror test. In the group test, rainbow trout were also bolder than both brown trout types, despite rainbow trout being shyer than both brown trout types when tested individually. However, a caveat of this test is that only the boldest fish was recorded in each case, rather than every fish in the group. These results may reflect an enhanced competitive drive influenced by the process of domestication, with aggression shown to be increased in hatchery but not wild rainbow trout fry previously [54], or a reduction in the stress response of individuals leading to bolder behaviour. Rainbow trout are often released or escape in large numbers, so these results may mean that boldness may depend on the densities of introduced individuals. Evaluating how the density of fish affects the boldness of individuals and their vulnerability to predation is critical to assess the likelihood of survival of mass released and escaped exotic trout.

High functional responses of invaders compared to natives are excellent predictors of high ecological impact [23,26,55-57], and our findings corroborate this, since the generally more impactful invader, the brown trout [33], exhibits a higher functional response than the rainbow trout. Although this comparative functional response experiment contradicted the results of the feeding test, which showed no differences between the three fish types, functional responses are better at assessing resource use [27]. The degree of impact was lessened when encountering a novel food source, suggesting that fish fed primarily on pellets may have a lesser impact when escaping into new environments with different food sources. Additionally, however, diploid brown trout showed a greater increase in functional response relative to the other fish types when exposed to a non-novel compared to a novel food source, suggesting that they may have a greater capacity for learning new food sources in the wild, contributing to their greater success and impact.

The importance of the behavioural variables in predicting impact is less clear, due to the contrasting results for boldness in the individual versus group conditions. Poorer management of energy reserves in escaped rainbow trout due to high aggression and activity may lead to excess mortality in the wild, leading to lower abundances and consequently low field impact. The increased activity may help to explain why in some studies rainbow trout is found to invade a wider range than brown trout [38], despite having a smaller impact on natives.

Triploid brown trout were significantly less active and less bold than the diploid brown trout. This was evident from the significantly fewer line crosses during the open field trial and novel object test, more time spent in the perimeter in the OFT, fewer shelter crosses, and less time outside the shelter. More casual observations were also made of erratic behaviour by the triploid trout, with darting movements within the arena. There has been extensive comparison of physiology between diploid and triploid fishes, with significant differences found in stress and disease resistance [42]. For example, triploid Atlantic salmon have reduced gill surface area, potentially impacting ventilation, with triploids also having lower respiratory efficiency than diploids [40, 58], triploids also do not deal well with chronic stress [59]. A reduced

ability to ventilate may have been a contributing factor to the differences in behaviour observed and, by reducing their activity, triploids may have been more effectively managing their own energy reserves. Alternatively, Atlantic salmon of differing ploidy showed found no difference in stress responses ^[60], therefore the different behaviour of the triploid brown trout may reflect cognitive differences compared to diploids. Previously no significant difference has been found between mixed-sex diploid and all-female triploid brown trout in terms of performance and survival when stocked, suggesting that in the wild any behavioural differences between the two varieties may have minimal consequences ^[41].

A behavioural syndrome of activity with boldness and aggression in both diploid and triploid brown trout but not in rainbow trout suggests that either this combination of traits has been more advantageous in the brown trout than in the rainbow trout lineage, or that there has been a greater disruptive effect of domestication on the association between these traits in rainbow trout. The stronger correlation in triploid brown trout, as well as an association between boldness and aggression within this syndrome, is surprising, since it would be expected that triploidy may have a more disruptive effect on any associations between these behaviours. Instead, it may be that triploid trout, due to their differences in physiology, present more extreme associations of behaviours.

A caveat of this analysis is that a wild analogue was not available for the species studied. Since all of the fish used in the experiment were domesticated strains it is not possible to disentangle the species and ploidy-level differences in behaviour from those due to the domestication process. Many of the successful invasions of both species worldwide occurred several decades ago, and would have involved strains much closer in phenotype to the wild source strains. Additionally, many trout are released at an age much older than the fry stage when they are even more habituated to the farm environment and so may be less likely to adapt in the wild. The changes to behaviour over time may also differ between the three trout types, meaning that the trends observed here in fry may not be the same for older fish. Mass escapes of all age groups do, however, occur, as evidenced by a mass escape of over 300 000 rainbow trout into the River Strule, Northern Ireland from a fish farm comprising all age classes of fish ^[61]. Despite these caveats, our findings suggest that the differential success of rainbow trout compared to brown trout may be partially explained by differences in the behaviour between the two fish species, possibly due to different stress responses. The findings that domesticated rainbow trout are shyer and have lower functional responses than brown trout may help to explain the lack of success and ecological impact of rainbow trout, especially in areas like the British Isles where the native wild brown trout is likely to be far better adapted to the local conditions, and so may limit invasion potential of the rainbow trout. Brown trout is typically seen as a safer option for stocking in Europe compared with rainbow trout since it is native to the area, however, with a higher functional response and greater success as an invader elsewhere, regular stocking may in fact be more detrimental to the environment compared with rainbow trout stocking, something which fisheries managers may wish to take into account. Alternatively, the greater activity and aggression of rainbow trout may mean that they are more disruptive through other means, such as disturbing native fish and attracting predators.

Our results demonstrate the value in combining behaviour tests with comparative functional response tests to evaluate the risks posed by invasive species, and to explore the reasons for differences in survival and impact. Furthermore, we show that these tests can even highlight significant differences between two highly domesticated species with a similar invasion history. Further research measuring differences in cortisol levels of both domesticated species under these experimental conditions would be useful, as well as a comparison between wild-type rainbow trout and brown trout with their respective domesticated relatives. Inclusion of triploid rainbow trout into the comparison would also be advantageous (and was not possible for this study) since they are also released into the wild. Changes in temperature and other environmental variables may also modify the behaviour of these species affecting their impact^[62], therefore performing these experiments under different temperature treatments would also be advantageous. This additional work would help to determine the relative importance of the processes of domestication and natural selection in shaping the differences between these species, and in combination with field-based studies would further help to elucidate the reasons behind the differences in the relative success and impacts of rainbow trout and brown trout as invasive species.

Methods

Collection and maintenance

Diploid rainbow trout fry (mean mass \pm SE: $0.418 \pm 0.0325\text{g}$), diploid brown trout fry ($0.602 \pm 0.0563\text{g}$), and triploid brown trout fry ($0.470 \pm 0.0311\text{g}$) were acquired from Movanager Fish Farm, Northern Ireland. Each fish type was acquired in a single batch of 100 fish approximately 8 weeks after the official hatch date of each type, between February to April 2019. We chose to age-match rather than size-match since we were interested in testing the fry at the same developmental stage with the same experience, however, we also used mass of fish in our statistical modelling of all behaviour variables to control for any size-effects. This method in which fish size is not controlled has been used in other studies comparing personality between species^[63]. Fry were moved to the Queen's University Belfast Medical Biology Centre, where they were held in a 12°C laboratory with a 12:12 light regime, and given three days to acclimate before trials commenced, with complete water changes every two days. Fry were housed in two holding tanks ($39.5 \times 25 \times 27\text{cm}$) with approximately 50 fish in each tank for the duration of the experiment and were fed *ad libitum* twice daily with INICIO Plus 0.5mm food pellets from the fish farm. Morning feeding took place after four fish had been selected for the day's experiments so that fish used in experiments were starved from approximately 6pm the previous day.

Ethical statement

Fish were kept in densities lower than those in the fish farm from which they were acquired (fish farm: approx. 41,269 – 61,904 fish per m^3 , our tanks: 1,872 fish per m^3) and, during transfer between the holding tanks and the experimental arenas, the time spent out of the water was minimised to less than 10s. This work did not fall under the definition of regulated procedures as per the UK Animals (Scientific Procedures) Act 1986, however, all experiments adhered to UK regulations and institutional ethical

approval was granted by the Queen's University Belfast, School of Biological Sciences Research Ethics Committee, and experiments were also conducted following recommendations in ARRIVE guidelines. After the end of the study, fish were returned to Movanager fish farm and kept isolated from the other fish on the farm for a week to prevent possible transfer of disease.

Behavioural trials

Each trial involved four fish being chosen at random from a holding tank, with each placed in 2L of dechlorinated tap water (previously oxygenated overnight and until usage in trials) in one of four white buckets (henceforth "arenas"), surrounded by cardboard screens to ensure shading from direct light. All four fish were videoed from above simultaneously during each trial. At the bottom of each arena two concentric circles marked out three regions: the perimeter, outer ring and central ring (Supplementary Figure S1). After introduction to the arenas on each day, fry were given a 15-minute adaptation period. This adaptation period was filmed for all rainbow trout, a subset of diploid brown trout and all triploid brown trout and served as an Open Field Test (OFT) within the study. After this OFT, one of our three additional behaviour tests (i.e. novel object, shelter or mirror tests) began, with the order of the trials on each day alternating.

Each part of each behaviour test consisted of a 15-minute recorded period. After fish were disturbed by transitions between each test, they were left to recover for 15 minutes before beginning the next test. The OFT and mirror test consisted of only one 15-minute part, but the novel object and shelter tests were further subdivided into two components, each filmed for 15 minutes. After all behaviour tests were completed all four fish were subjected to a feeding trial, then weighed. Finally, each fish was placed into one of the original arenas to form a group of four conspecifics, with the group then filmed for an OFT, and the two components of the novel object test. See Supplementary Figure S2 for a full flow chart of the behaviour tests.

Video analysis

All 15-minute tests (detailed below) were trimmed to 10 minutes prior to analysis, using Shotcut video editing software, to make analyses of all videos feasible in the time available, and because preliminary viewings didn't show dramatic changes in behaviour in the final five minutes. The OFT was cut to start immediately after the final disturbance to each of the fish. All other tests were trimmed to start 10 seconds after the final disturbance to each of the fish. BORIS was used to perform all behaviour analyses [64]. The position of each fish was determined by the location of the fish's head. Data were compiled using R Studio version 1.3.1093 [65].

Open field test

Fish were released into the arena at the start of the day and their reaction to the new environment was recorded. For each video, the percentage time spent swimming versus resting and the total number of line crosses between the locations in the arena (whether in the perimeter, outer ring or central ring) where

recorded as measures of activity. The latency to start swimming and the percentage time in each location were also recorded as measures of boldness. Where fish stayed still for the entirety of the video, latency was recorded as 600s (the total length of the video).

Disturbance/novel object test

This paired test consisted of two 15-minute components. In the first component (disturbance test), a blue plastic airline splitter was quickly tapped on the surface of the water for each arena; this was to mimic the disturbance to the fish of the novel object being introduced into the arena. In the second component of the test (novel object test), the same object was lowered into the central circle of the arena. For both tests, activity was measured as the total number of line crosses between arena locations. The latency to enter the central ring and the percentage time spent in each location of the arenas were recorded as measures of boldness. In cases where the fish did not enter the central ring during the trial, the latency was recorded as 600s (the length of the video).

Shelter/predation test

This paired test also involved two 15-minute components. Cardboard screens were removed prior to the 15-minute acclimation period before the two parts. For both parts a cardboard sheet was placed over 50% of the area of each arena (Supplementary Figure S1). In the first part (shelter test), fry were filmed in the presence of the shelter and with no other disturbance. In the second part (predation test), a person was present throughout the test and waved their hands above the arenas every 30 seconds for approximately 5 seconds to simulate a predation threat. Fish were recorded as either under or outside the shelter. The number of transitions between inside and outside the shelter were recorded as a measure of activity, and percentage time outside the shelter recorded as a measure of boldness.

Mirror test

A square mirrored tile (15 x 15cm) was lowered into each arena and fixed against the side of each arena with adhesive putty (Supplementary Figure S1). During video analysis, an active zone adjacent to the mirror was defined as between the mirror and the line outlining the outer ring. The percentage time spent within or outside the active zone was determined. In addition, the percentage of the time within the active zone spent doing the following three behaviours was also calculated: active behaviour (swimming while oriented to the mirror), passive behaviour (swimming away from the mirror), and freeze behaviour (lying still). The latency to start swimming alongside the mirror was also recorded as a separate measure of aggression.

Feeding test

Fry were moved from their arenas, and placed in transparent food containers (14.5 x 9.5 x 4 cm dimensions and volume 400ml) with 10 bloodworms (*Chironomidae* spp.). Trials ran for four hours, with the number of alive prey remaining counted afterwards. Fry were then weighed using electronic scales (Table 1).

Table 1 List of response variables from each of the behaviour tests, and the behavioural descriptors for each variable.

Test	Response variable	Behaviour
Open Field test	latency to swim	Boldness
	total line crosses	Activity
	percentage time swimming	Activity
	percentage time in perimeter	Boldness
	percentage time in outer ring	Boldness
	percentage time in central ring	Boldness
Disturbance and novel object test	latency to approach	Boldness
	total line crosses	Activity
	percentage time in perimeter	Boldness
	percentage time in outer ring	Boldness
	percentage time in central ring	Boldness
Shelter and predation test	percentage outside shelter	Boldness
	shelter crosses	Activity
Mirror	latency to begin swimming along mirror	Aggression
	percentage in active zone	Aggression
	percentage time swimming in active zone	Aggression
	percentage time passive in active zone	Aggression
	percentage time frozen in active zone	Aggression
Group test	latency of first fish to approach	Boldness

Group test

The four fish involved in the simultaneous trials each day were introduced into a single arena. After all four fish had been introduced, the cardboard screens were placed around the arena and the fish were left to acclimatise for 15 minutes. The disturbance test and novel object test were then conducted as for the individual tests, this time using a small, purple, plastic figurine as the novel object. The latency of the fastest fish to approach the central circle was then recorded using BORIS for this test ^[64].

Functional response trials

The functional response of all three trout types to bloodworms as prey were measured, with two levels of experience also tested. Fry that were naïve to bloodworms (i.e. had not been used in the behavioural trials) were used to measure the functional response to “novel” prey. Fry with prior experience of bloodworms from the behavioural trials were used to measure the functional response to “non-novel” prey. Both groups of fry were kept in separate holding tanks and starved overnight (approximately 15 hours). Individual fry were placed in arenas (14.5 x 9.5 x 4 cm dimensions and volume 400ml) which were made visually uniform by having masking tape wrapped around the outside. Lids were placed on top of these containers to prevent escape of fish and green plastic sheets were placed around the arenas to prevent additional visual stimuli. Six densities of prey were used (1, 2, 4, 8, 16 and 32: n = 3 per prey density per trout type per experience level) and fry were added to these arenas and allowed to feed for four hours (similar design to Alexander et al. 2014), with the number of alive prey counted afterwards. Controls were performed (n = 3 per prey density) with the same experimental conditions but in the absence of fry, to quantify prey mortality for any other reasons.

Statistical analyses

Individual tests

Because preliminary modelling revealed heteroscedasticity in some of the residuals for the models, for each of the response variables from the behaviour tests, model selection was performed following the protocol outlined in Zuur et al (2008) ^[66]. All models were fitted using the nlme package ^[67] in R ^[65]. All data compilation was performed using the dplyr package ^[68] and plots generated using ggplot2 ^[69].

First, a linear model was fitted using two explanatory variables: type of fish (i.e. diploid rainbow, diploid brown, or triploid brown) and mass of fish. For the disruption/novel object and shelter/predation tests the component of the test was also included as a factor (i.e. the first versus the second 15-minute segment of the test) with an interaction term between the test component and the type of fish (Table 1). Likelihood ratio tests were used to compare this model structure with models containing a random factor of Arena. Finally, since heterogeneity was observed across fish type for several response variables, a variance structure varying by fish type was also included and a likelihood ratio test used to determine its significance. In total, 19 response variables were modelled in this way (Table 1). All proportion data were arc-sine square-root transformed and number of line crosses \log_{10} transformed prior to modelling for better distribution of residuals in resulting models. Latency (+ 1) data were also \log_{10} -transformed, except for the novel object test where residuals for the latency models were more normally distributed using a gaussian distribution. The dredge function ^[70] was used on all global models (after random effect and variance structure were selected) to determine the most parsimonious in all cases, with the top model reported by dredge being determined the most parsimonious model. Contrasts across different factor levels among any interactions included in the final models were calculated using the emmeans package ^[71].

Feeding test

The number of bloodworms eaten by the fish was modelled with respect to the type of fish, fish mass and the response variables from the previous behaviour tests for which a significant difference was found between the fish types. Model selection was carried out using the Zuur et al (2008) protocol as above.

Correlational analysis

For each fish type, Pearson's correlations were performed for a selection of variables describing activity, boldness and aggression. Only combinations of different behaviour types were considered in the analysis, i.e. bold traits with active traits, as well as correlations between these variables and fish mass and number of bloodworms eaten. Additionally, only correlations which involved combinations of different tests were considered, since some variables are not independent within each test (i.e. latency to approach object and number of line crosses in the novel object test).

Group test

The latency of the first fish to approach the central circle was modelled with respect to fish type, whether the disturbance or novel object test, and the mean mass of the fish in the test. An interaction term between fish type and type of test was also included in the most complex model. Model selection was carried out using the protocol as above, with date tested as a random effect.

Functional response trials

Functional Responses (FR) were modelled using the 'frair' package^[72]. The FR curve types (Type I, II or III^[27]) were derived through logistic regression of the proportion of prey consumed as a function of prey density offered^[73]. Significantly negative first-order terms indicate hyperbolic Type II curves, whereas significantly positive first-order terms followed by significantly negative second-order terms indicate sigmoidal Type III curves. Rogers' random predator equation was used to model FRs since prey were not replaced as they were consumed^[74]:

$$N_e = N_0(1 - \exp(-a(N_e h - T))) \quad (1)$$

where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack constant, h is the handling time and T is the total experimental period (i.e. four hours). Maximum feeding rates ($1/h$) were calculated under each treatment group. The *Lambert W* function was used to solve the random-predator equation^[75]. We generated 95% confidence intervals around FR curves using non-parametric bootstraps ($n = 2000$).

Inter-rater repeatability test

JWED analysed all mirror test videos, JWED and CM analysed all OFT videos and student assistants SD, CAN and CM analysed all remaining tests. For those tests in which more than one person was involved in the analysis, 10% of videos were analysed by all observers. A statistical model including arena, observer and fish type as explanatory variables was run for each variable. In each case there was no significant

effect of observer, except for one: the number of line crosses in the paired disturbance/novel object test. Because there was a significant effect of observer in this case, the modelling for this variable was repeated including observer as an explanatory variable, but there was no effect on the significance of the other variables in the model.

Declarations

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Author contributions

Original conception of the project was by JWED, with experimental design by JWED, GA and CLOM. JWED, CLOM, SD and CN performed all video analyses. CLOM conducted the statistical analyses and wrote the majority of the manuscript text. All authors contributed to the development and writing of this manuscript.

Additional information

Competing interests: The authors declare that they have no competing interests.

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

References

1. Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. Integrating animal temperament within ecology and evolution. *Biol. Rev.* **82**, 291–318 (2007).
2. Millot, S. *et al.* Assessment of genetic variability of fish personality traits using rainbow trout isogenic lines. *Behav. Genet.* **44**, 383–393 (2014).
3. Weis, J. & Sol, D. Behaviour and the Invasion Process. in *Biological Invasions and Animal Behaviour* 5–116 (Cambridge University Press., 2016).
4. Sih, A., Bell, A. & Johnson, J. C. Behavioral syndromes: An ecological and evolutionary overview. *Trends Ecol. Evol.* **19**, 372–378 (2004).
5. Sneddon, L. U. The bold and the shy: Individual differences in rainbow trout. *J. Fish Biol.* **62**, 971–975 (2003).

6. Conrad, J. L., Weinersmith, K. L., Brodin, T., Saltz, J. B. & Sih, A. Behavioural syndromes in fishes: A review with implications for ecology and fisheries management. *J. Fish Biol*, **78**, 395–435 (2011).
7. Mowles, S. L., Cotton, P. A. & Briffa, M. Consistent crustaceans: The identification of stable behavioural syndromes in hermit crabs. *Behav. Ecol. Sociobiol*, **66**, 1087–1094 (2012).
8. Bell, A. M. Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *J. Evol. Biol*, **18**, 464–473 (2005).
9. Bourne, G. R. & Sammons, A. J. Boldness, aggression and exploration: evidence for a behavioural syndrome in male pentamorphic livebearing fish, *Poecilia parae*. *AAFL Bioflux* **39–50** (2008).
10. Carter, A. J. & Feeney, W. E. Taking a comparative approach: Analysing personality as a multivariate behavioural response across species. *PLoS One* **7**, (2012).
11. Bolnick, D. I. *et al.* Why intraspecific trait variation matters in community ecology. *Trends Ecol. Evol*, **26**, 183–192 (2011).
12. Start, D. & Gilbert, B. Predator personality structures prey communities and trophic cascades. *Ecol. Lett*, **20**, 366–374 (2017).
13. Holway, D. A. & Suarez, A. V. Animal behavior: an essential component of invasion biology. *TREE*, **14**, 328–330 (1999).
14. Chapple, D. G., Simmonds, S. M. & Wong, B. B. M. Can behavioral and personality traits influence the success of unintentional species introductions? *Trends Ecol. Evol*, **27**, 57–64 (2012).
15. Cote, J., Fogarty, S., Weinersmith, K., Brodin, T. & Sih, A. Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proc. R. Soc. B Biol. Sci.* **277**, 1571–1579 (2010).
16. Myles-Gonzalez, E., Burness, G., Yavno, S., Rooke, A. & Fox, M. G. To boldly go where no goby has gone before: Boldness, dispersal tendency, and metabolism at the invasion front. *Behav. Ecol*, **26**, 1083–1090 (2015).
17. Mutascio, H. E., Pittman, S. E. & Zollner, P. A. Investigating movement behavior of invasive Burmese pythons on a shy–bold continuum using individual-based modeling. *Perspect. Ecol. Conserv*, **15**, 25–31 (2017).
18. Chuang, A. *Living life on the edge: The role of invasion processes in shaping personalities in a non-native spider species* (The University of Tennessee, Knoxville, 2019).
doi:10.1017/CBO9781107415324.004
19. Blackburn, T. M. *et al.* A proposed unified framework for biological invasions. *Trends Ecol. Evol*, **26**, 333–339 (2011).
20. Pintor, L. M., Sih, A. & Kerby, J. L. Behavioral correlations provide a mechanism for explaining high invader densities and increased impacts on native prey., **90**, 581–587 (2009).
21. Petren, K. & Case, T. J. An experimental demonstration of exploitation competition in an ongoing invasion., **77**, 118–132 (1996).
22. Wright, T. F., Eberhard, J. R., Hobson, E. A., Avery, M. L. & Russello, M. A. Behavioral flexibility and species invasions: The adaptive flexibility hypothesis. *Ethol. Ecol. Evol*, **22**, 393–404 (2010).

23. Dick, J. T. A. Role of behaviour in biological invasions and species distributions; lessons from interactions between the invasive *Gammarus pulex* and the native *G. duebeni* (Crustacea: Amphipoda). *Contrib. to Zool*, **77**, 91–98 (2008).
24. Dick, J. T. A. *et al.* Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. *J. Appl. Ecol*, **54**, 1259–1267 (2017).
25. Dick, J. T. A., Elwood, R. W. & Montgomery, W. I. The behavioural basis of a species replacement: differential aggression and predation between the introduced *Gammarus pulex* and the native *G. duebeni celticus* (Amphipoda). *Behav. Ecol. Sociobiol*, **37**, 393–398 (1995).
26. Dick, J. T. A. *et al.* Ecological impacts of an invasive predator explained and predicted by comparative functional responses. *Biol. Invasions*, **15**, 837–846 (2013).
27. Dick, J. T. A. *et al.* Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. *Biol. Invasions*, **16**, 735–753 (2014).
28. Iacarella, J. C., Dick, J. T. A. & Ricciardi, A. A spatio-temporal contrast of the predatory impact of an invasive freshwater crustacean. *Divers. Distrib*, **21**, 803–812 (2015).
29. Toscano, B. J. & Griffen, B. D. Trait-mediated functional responses: Predator behavioural type mediates prey consumption. *J. Anim. Ecol*, **83**, 1469–1477 (2014).
30. MacCrimmon, H. R. World distribution of rainbow trout (*Salmo gairdneri*): further observations. *J. Fish. Res. Board Canada*, **28**, 663–704 (1971).
31. MacCrimmon, H. R., Marshall, T. L. & Gots, B. L. World distribution of brown trout, *Salmo trutta*: further observations. *J. Fish. Res. Board Canada*, **27**, 811–818 (1970).
32. Crawford, S. S. & Muir, A. M. Global introductions of salmon and trout in the genus *Oncorhynchus*: 1870–2007. *Rev. Fish Biol. Fish*, **18**, 313–344 (2008).
33. Crowl, T. A., Townsend, C. R. & McIntosh, A. R. The impact of introduced brown and rainbow trout on native fish: the case of Australasia. *Rev. Fish Biol. Fish*, **241**, 217–241 (1992).
34. Hasegawa, K. Invasions of rainbow trout and brown trout in Japan: A comparison of invasiveness and impact on native species. *Ecol. Freshw. Fish*, **29**, 419–428 (2020).
35. Cambray, J. A. The global impact of alien trout species—a review; with reference to their impact in South Africa. *African J. Aquat. Sci*, **28**, 61–67 (2003).
36. Dunham, J. B., Wheeler, A. & Rosenberger, A. Assessing the consequences of nonnative trout in headwater ecosystems in western North America. *Fisheries*, **29**, 37–41 (2004).
37. Fausch, K. D., Taniguchi, Y., Nakano, S., Grossman, G. D. & Townsend, C. R. Flood disturbance regimes influence rainbow trout invasion success among five holarctic regions. *Ecol. Appl*, **11**, 1438–1455 (2001).
38. Young, K. A. *et al.* A trial of two trouts: Comparing the impacts of rainbow and brown trout on a native galaxiid. *Anim. Conserv*, **13**, 399–410 (2010).
39. Lincoln, R. F. & Scott, A. P. Production of all-female triploid rainbow trout., **30**, 375–380 (1983).

40. Maxime, V. The physiology of triploid fish: Current knowledge and comparisons with diploid fish. *Fish Fish*, **9**, 67–78 (2008).
41. Chatterji, R., Longley, D., Sandford, D., Roberts, D. & Stubbing, D. Performance of stocked triploid and diploid brown trout and their effects on wild brown trout in UK rivers. (2008).
42. Benfey, T. J. The physiology and behavior of triploid fishes. *Rev. Fish. Sci*, **7**, 39–67 (1999).
43. Carter, C. G. *et al.* Food consumption, feeding behaviour, and growth of triploid and diploid Atlantic salmon, *Salmo salar* L., parr. *Can. J. Zool*, **72**, 609–617 (1994).
44. Øverli, Å., Pottinger, T. G., Carrick, T. R., Øverli, E. & Winberg, S. Differences in behaviour between rainbow trout selected for high- and low-stress responsiveness. *J. Exp. Biol*, **205**, 391–395 (2002).
45. Adriaenssens, B. & Johnsson, J. I. Learning and context-specific exploration behaviour in hatchery and wild brown trout. *Appl. Anim. Behav. Sci*, **132**, 90–99 (2011).
46. Näslund, J. & Johnsson, J. I. State-dependent behavior and alternative behavioral strategies in brown trout (*Salmo trutta* L.) fry. *Behav. Ecol. Sociobiol*, **70**, 2111–2125 (2016).
47. Mortensen, E. Density-dependent mortality of trout fry (*Salmo trutta* L.) and its relationship to the management of small streams. *J. Fish Biol*, **11**, 613–617 (1977).
48. Armstrong, J. D. & Nislow, K. H. Critical habitat during the transition from maternal provisioning in freshwater fish, with emphasis on Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). *J. Zool*, **269**, 403–413 (2006).
49. Walsh, R. N. & Cummins, R. A. The open-field test: A critical review. *Psychol. Bull*, **83**, 482–504 (1976).
50. Adriaenssens, B. & Johnsson, J. I. Shy trout grow faster: exploring links between personality and fitness-related traits in the wild. *Behav. Ecol*, **22**, 135–143 (2010).
51. Sneddon, L. U., Braithwaite, V. A. & Gentle, M. J. Novel object test: examining nociception and fear in the rainbow trout. *J. Pain*, **4**, 431–440 (2003).
52. Adriaenssens, B. *Individual variation in behaviour: personality and performance of brown trout in the wild* (University of Gothenburg, 2010).
53. Sloman, K. A., Metcalfe, N. B., Taylor, A. C. & Gilmour, K. M. Plasma cortisol concentrations before and after social stress in rainbow trout and brown trout. *Physiol. Biochem. Zool*, **74**, 383–389 (2001).
54. Berejikian, B. A., Mathews, S. B. & Quinn, T. P. Effects of hatchery and wild ancestry and rearing environments on the development of agonistic behavior in steelhead trout (*Oncorhynchus mykiss*) fry. *Can. J. Fish. Aquat. Sci*, **53**, 2004–2014 (1996).
55. Laverty, C. *et al.* Assessing the ecological impacts of invasive species based on their functional responses and abundances. *Biol. Invasions*, **19**, 1653–1665 (2017).
56. Alexander, M. E., Dick, J. T. A., Weyl, O. L. F., Robinson, T. B. & Richardson, D. M. Existing and emerging high impact invasive species are characterized by higher functional responses than natives. *Biol. Lett.* **10**, (2014).

57. Dickey, J. W. E., Cuthbert, R. N., Steffen, G. T., Dick, J. T. A. & Briski, E. Sea freshening may drive the ecological impacts of emerging and existing invasive non-native species. *Divers. Distrib*, **27**, 144–156 (2021).
58. Sadler, J., Pankhurst, P. M. & King, H. R. High prevalence of skeletal deformity and reduced gill surface area in triploid Atlantic salmon (*Salmo salar* L.), **198**, 369–386 (2001).
59. Benfey, T. J. & Biron, M. Acute stress response in triploid rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*), **184**, 167–176 (2000).
60. Sadler, J., Pankhurst, N. W., Pankhurst, P. M. & King, H. Physiological stress responses to confinement in diploid and triploid Atlantic salmon. *J. Fish Biol*, **56**, 506–518 (2000).
61. Whelan, K. Assessing and mitigating the impact of a major rainbow trout escape on the wild salmon and trout populations of the Mourne river system, Northern Ireland. (2017).
62. Shelton, J. *et al.* Temperature mediates the impact of non-native rainbow trout on native freshwater fishes in South Africa's Cape Fold Ecoregion. *Biol. Invasions*, **20**, 2927–2944 (2018).
63. Michelangeli, M. *et al.* Sex-dependent personality in two invasive species of mosquitofish. *Biol. Invasions*, **22**, 1353–1364 (2020).
64. Friard, O. & Gamba, M. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol*, **7**, 1325–1330 (2016).
65. R Core Team. R: A language and environment for statistical computing. (2018).
66. Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. & Smith, G. M. Mixed Effects Models and Extensions in Ecology with R. *Springer*, <https://doi.org/10.1086/648138> (2008).
67. Bates, D., Mächler, M., Bolker, B. M. & Walker, S. C. Fitting linear mixed-effects models using lme4. *J. Stat. Softw*, **67**, 18637 (2015).
68. Wickham, H., François, R., Henry, L. & Müller, K. dplyr: A Grammar of Data Manipulation. R package version. *Media*, <https://doi.org/10.1007/978-0-387-98141-3> (2019).
69. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis* (Springer-Verlag, New York, 2016).
70. Barton, K. & MuMIn Multi-Model Inference 2020 (2020).
71. Lenth, R., Singmann, H., Love, J., Buerkner, P. & Herve, M. emmeans: estimated marginal means, aka least-squares means. R package version 1.5.2-1(2020).
72. Pritchard, D. frail: tools for functional response analysis. R package version 0.0.100(2017).
73. Juliano, S. A. Predation and functional response curves. in *Design and Analysis of Ecological Experiments* (eds. Scheiner, S. & Gurevitch, J.) Chap. 10(2001).
74. Rogers, D. Random search and insect population models. *J. Anim. Ecol*, **41**, 369–383 (1972).
75. Bolker, B. M. Rogers random predator equation: extensions and estimation by numerical integration. 1–20(2012).

Figures

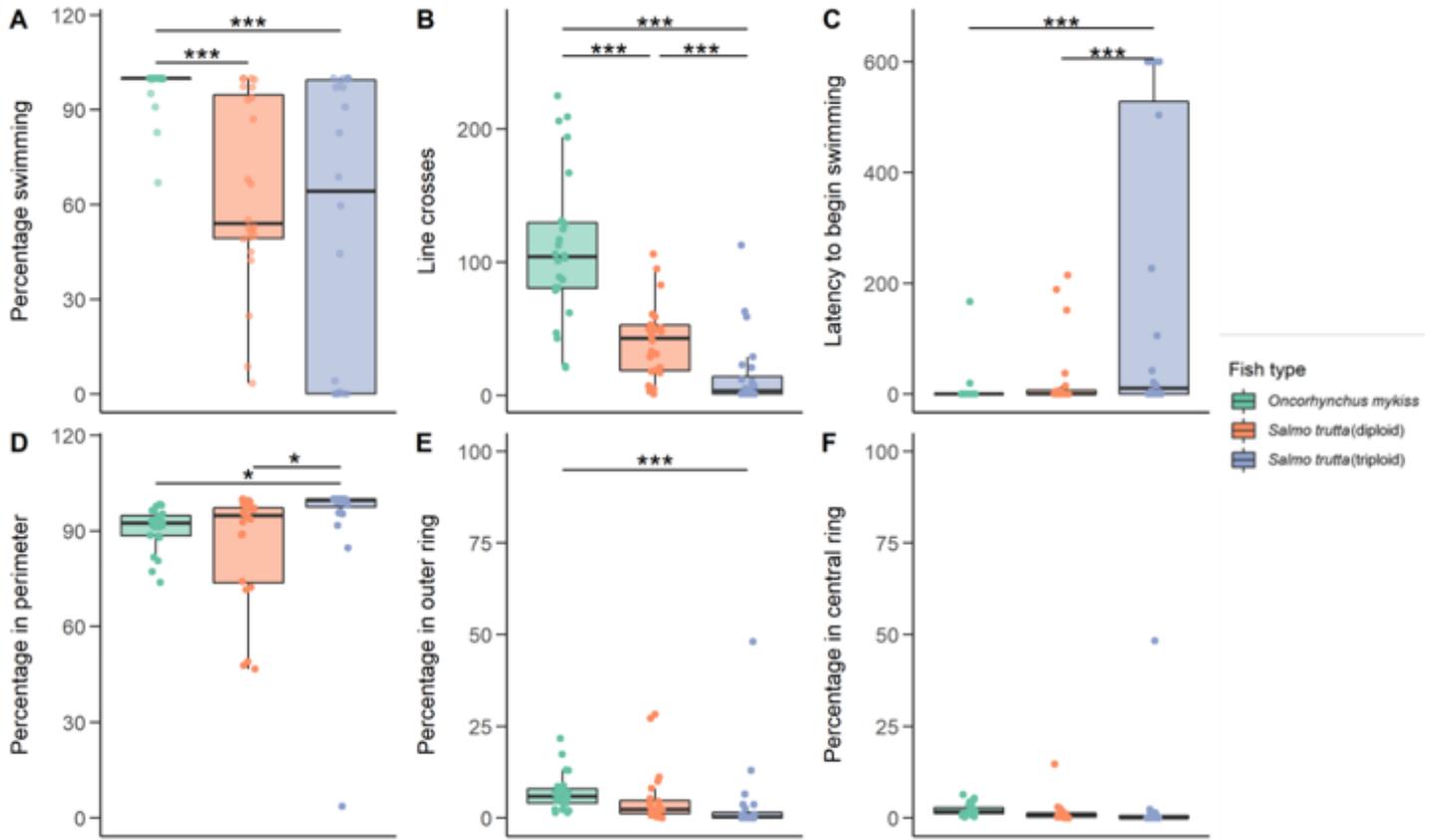


Figure 1

Boxplots showing the variables measured during the open field test, with median and interquartile range plotted and overlaid with raw data points. Asterisks show significance values. A: Percentage time spent swimming, B: Total number of line crosses, C: The latency to begin swimming from the start of the recording, D: Percentage time spent in the perimeter, E: Percentage time spent in the outer ring, F: Percentage time spent in the centre of the arena.

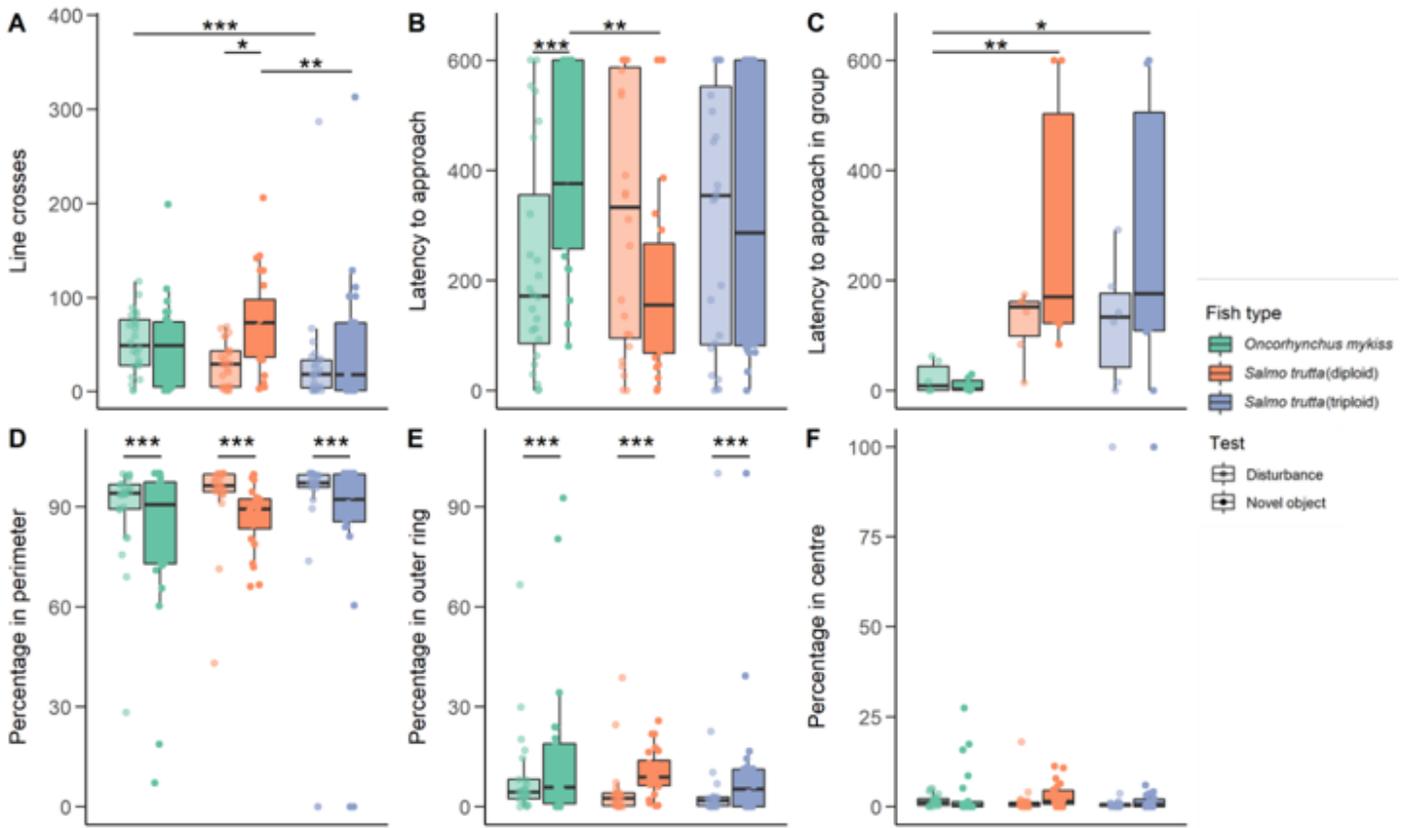


Figure 2

Boxplots showing the variables measured during the disturbance and novel object tests, including the group test, with median and interquartile range plotted and overlaid with raw data points. Asterisks show significance values. A: Total number of line crosses, B: The latency to begin swimming from the start of the recording in the individual tests, C: The latency to begin swimming from the start of the recording in the group tests, D: Percentage time spent in the perimeter, E: Percentage time spent in the outer ring, F: Percentage time spent in the centre of the arena.

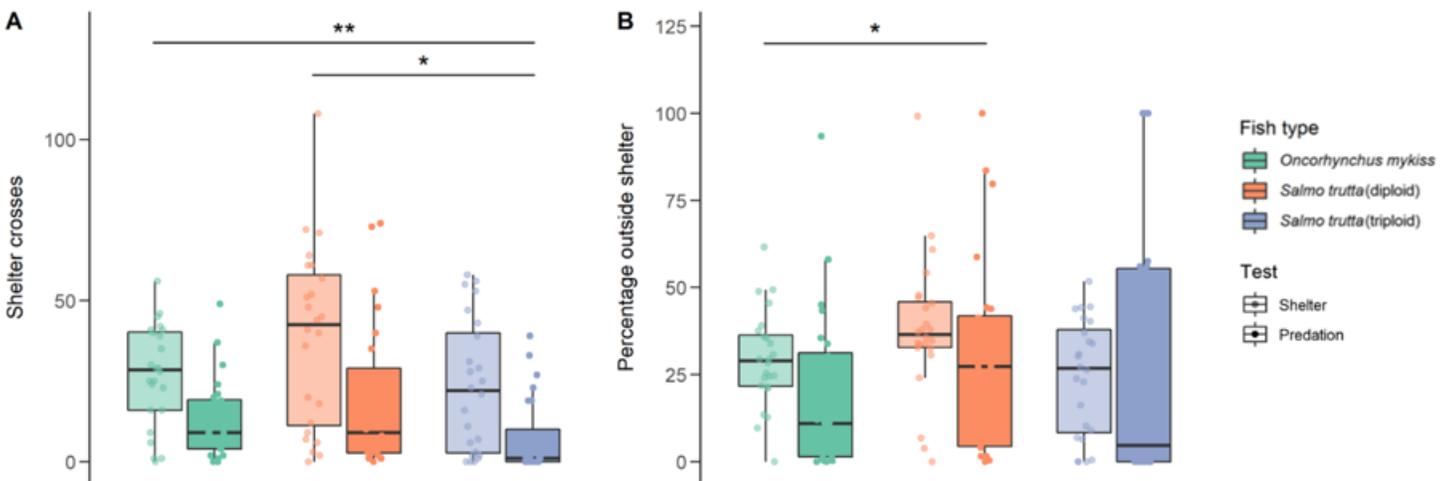


Figure 3

Boxplots showing the variables measured during the shelter and predation tests, with median and interquartile range plotted and overlaid with raw data points. Asterisks show significance values. A: Total number of shelter crosses, B: Percentage time spent outside the shelter.

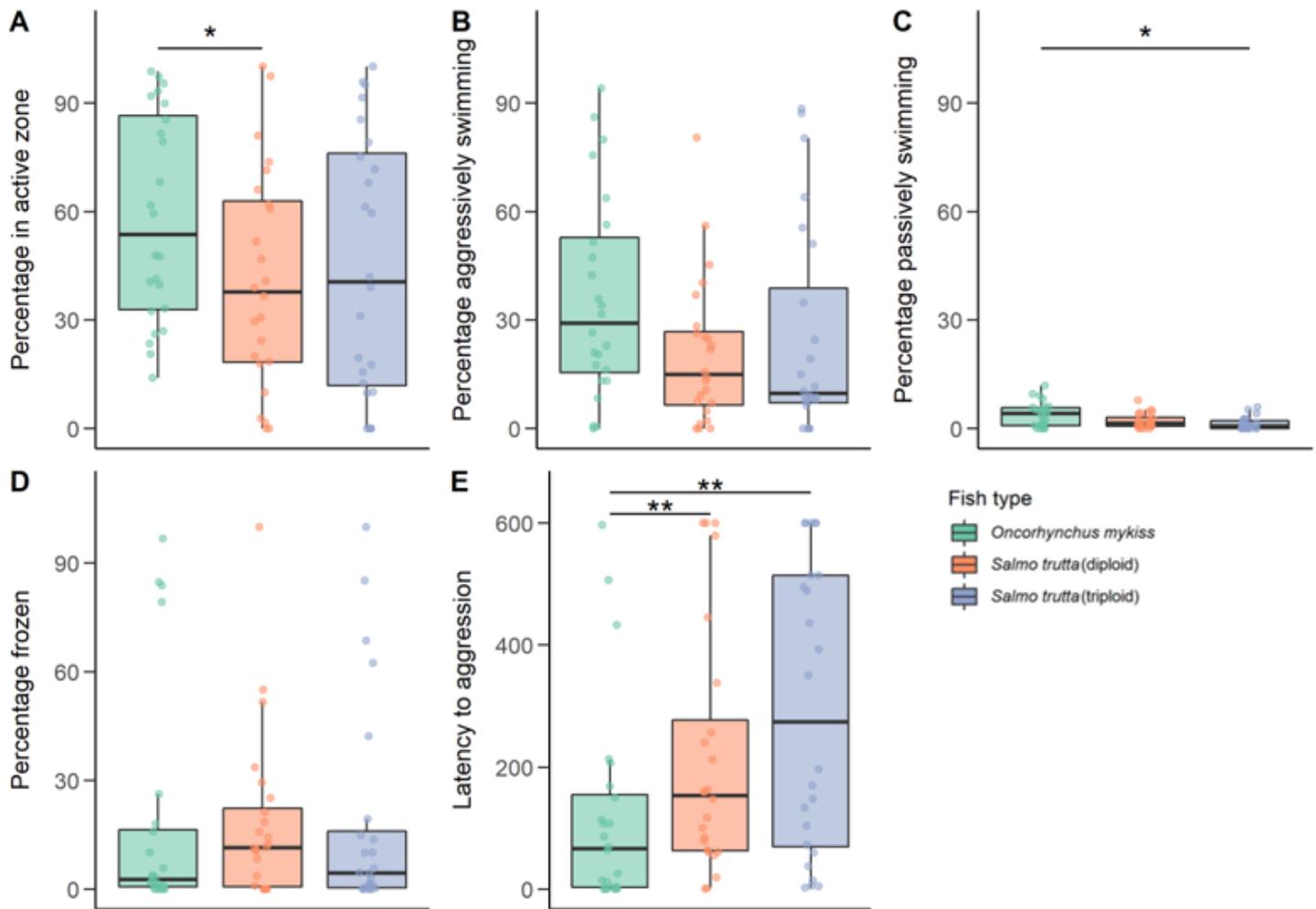


Figure 4

Boxplots showing the variables measured during the mirror test, with median and interquartile range plotted and overlaid with raw data points. Asterisks show significance values. A: Percentage time spent in the “active zone” adjacent to the mirror, B: Percentage of the time in the active zone spent aggressively swimming against the mirror, C: Percentage of the time in the active zone spent passively swimming away from the mirror, D: Percentage of the time in the active zone spent being still, E: Latency to initiate aggressive swimming against the mirror.

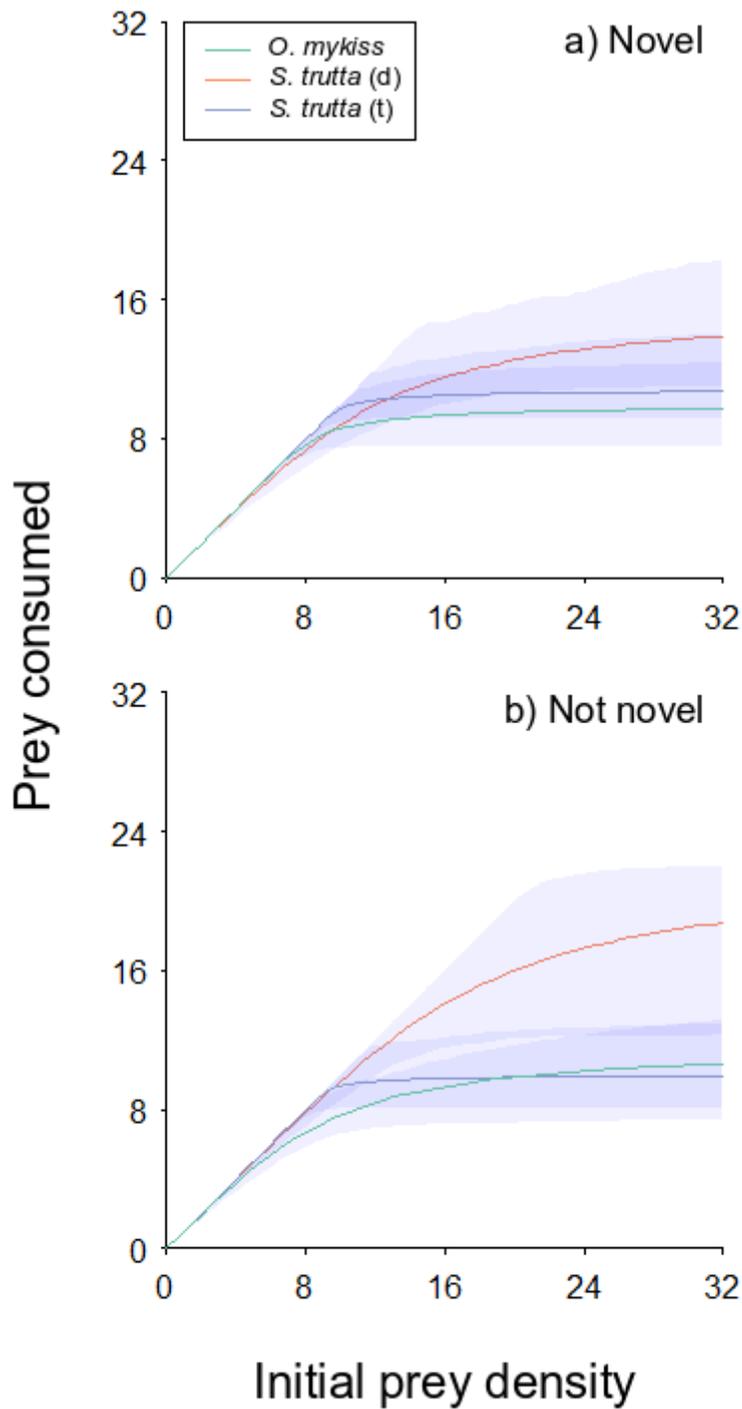


Figure 5

Functional Responses of diploid rainbow trout fry, diploid brown trout fry, and triploid brown trout fry under a) novel prey conditions (i.e. fry had never fed on live chironomid prey previously) and b) not novel conditions (i.e. fry had experienced live chironomid prey previously as part of the behaviour experiment). Clouds around lines represent bootstrapped 95 % confidence intervals.

Supplementary Files

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