On behavioural responses to smell and sight of alpine bullhead *Cottus poecilopus* Heckel for an allopatric and a sympatric population of brown trout *Salmo trutta* L.

Cand. scient. thesis in zoology
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Abstract

Freshwater sculpins and salmonids coexist in many streams throughout the Northern hemisphere, and often constitute an important component of stream ecosystems. Alpine bullhead *Cottus poecilopus* Heckel have been known to predate eggs and fry of brown trout *Salmo trutta* L., and also to function as a competitor to older brown trout for habitat and prey items. This study was designed to examine possible behavioural differences in activity level and positioning between a sympatric and an allopatric population of brown trout, when subjected to sight and smell of alpine bullhead. Sympatric and allopatric brown trout was collected from the same river system, and subjected to smell and sight of alpine bullhead under experimental conditions. Activity levels and positioning in three dimensions were recorded and analyzed, with the aid of digital video cameras.

When exposed to stimuli from alpine bullhead, neither brown trout population displayed changes in their positioning and activity level, statistically different from control experiments. Nevertheless, differences in behaviour between the brown trout populations were discovered. Allopatric brown trout changed their vertical position more than sympatric brown trout, in response to changes in their environment. In the same situations, there was larger variation between allopatric brown trout when it came to changes in horizontal and vertical positioning, than between the sympatric brown trout. The results from this study indicate that there is low level of aggression between alpine bullhead and brown trout, and that the behavioural differences discovered could be due to different degrees of intraspecific competition in the brown trout populations.

Introduction

Interactions between two species can take different forms. The term "competition" is used to describe an interaction between individuals in which one or more of the participants suffers a net loss of fitness, and none shows a net gain compared with values in the absence of the competitive interaction (Wootton 1998). "Interspecific competition" is used when these individuals come from different species, contrasting the term "intraspecific competition," which is used to describe competition within species. Interspecific competition is often highly asymmetric, and the consequences are thus often very different for the species involved (Begon et al. 1996).

To live in a competitive relationship with another species can affect a number of traits (Stearns 1992, Begon et al. 1996). For many species, behavioural repertoires play an important part in their response to competition. How a species distributes itself in the environment, and the level of evasiveness or aggressiveness toward competitors, are examples of such important behavioural responses (Krebs and Davies 1993, Drickamer et al. 1996). Studies of behavioural responses to competition can therefore give insight into the nature of the competition, as well as how strongly the species is influenced by this relationship.

Freshwater sculpins (genus *Cottus*) and salmonids are inhabitants in many of the same streams throughout the Northern hemisphere, and often constitute an important component of stream ecosystems (Petrosky and Waters 1975, Moyle 1977). The ecological relationship between cottids and salmonids has therefore come into focus in a number of studies, generating much debate on how the presence of cottids affects populations of salmonids.

Several studies have found that sculpins feed on salmonids eggs and fry (Gaudin and Caillere 2000, Hesthagen and Heggenes 2003, Tabor et al. 2004). Gaudin and Caillere discovered that the presence of sculpins affected displacement of brown trout *Salmo trutta* L. alevins in artificial channels. They suggested that the response had developed to avoid predation by sculpins, and that this behaviour might affect population dynamics (Gaudin and Caillere 2000).

Other studies have found cottids to be a competitor to salmonids for various resources (Gabler and Amundsen 1999, Holmen et al. 2003). There has been reports of an inverse relationship between density of brown trout and alpine bullhead *Cottus poecilopus* Heckel, a high degree of diet overlap and evidence of habitat displacement of brown trout in sympatry with alpine bullhead (Hesthagen and Heggenes 2003, Hesthagen et al. 2004). A Swedish study also found that the presence of alpine bullhead, and other fish species, affected both habitat use and life history traits in brown trout in the wild (Näslund et al. 1998).

On the other hand, there are studies claiming that the degree of predation and competition could be relatively weak (Straskraba et al. 1966, Petrosky and Waters 1975, Moyle 1977, Pihlaja et al. 1998, Jørgensen et al. 1999, Tomaro 2006). Some studies conclude that the varying densities of Atlantic salmon parr *Salmo salar* L. in different parts of a watercourse is due to different physical conditions, rather then the presence of cottids, and that there is a consistent segregation in food niche between alpine bullhead and Atlantic salmon parr (Jørgensen et al. 1999, Gabler et al. 2001).

Possible interspecific competition between brown trout and alpine bullhead appear to occur on a dynamic scale. Experimental studies under controlled conditions can therefore help illuminate the nature of the relationship between species (Hesthagen and Heggenes 2003).

As part of a project on the life-history variability of brown trout in forest streams, experiments have been conducted in a river-system in south-eastern Norway. The aim has been to test if allopatric populations and populations living with sympatry with alpine bullhead have evolved different early life-history traits (Olsen and Vøllestad 2001b). The density of brown trout is lower in sympatry than in allopatry in the study area. This reduced density probably leads to reduced intraspecific competition, and increased growth for sympatric trout. A laboratory mating-experiment with brown trout from this area showed significant differences in the early life-history traits between the populations (Olsen and Vøllestad 2001b). Sympatric fry grew more rapidly, and they also experienced lower mortality rates during first feeding than allopatric fry. A capture-mark-recapture study reported that the growth rate of brown trout in sympatry with alpine bullhead was lower than for allopatric brown trout, if correcting for variation in the density of brown trout, temperature, season and fish mass (Vøllestad et al. 2002). It was suggested that the findings could possibly indicate behavioural differences between trout living in allopatry or sympatry with alpine bullhead.

During experiments it was observed that fry from the sympatric population seemed to have a higher level of activity during feeding than fry from the allopatric population (Olsen and Vøllestad 2001b). This was thought to increase their survival rate, since active fish are more likely to acquire suitable territory, and avoid predation from the alpine bullhead. The survival of brown trout is also slightly lower in sites with the presences of alpine bullhead, than in sites without (Olsen and Vøllestad 2001a). These results further indicate competition between brown trout and alpine bullhead. It has also been suggested that the alpine bullhead could influence maturity responses in brown trout through either growth rates, survival rates, or both (Olsen and Vøllestad 2005). Another study conducted in the same area found brown trout in age class 0+ to 3+ and alpine bullhead to have rather similar diets (Holmen et al. 2003). Sympatric brown was suggested to have a different foraging behaviour, feeding more frequently on prey items high in the water column and on the surface, than allopatric individuals. Brown trout in these positions are hypothesized to be at greater predation risk, and this was proposed as an explanation for the lower population densities in sympatric populations.

The suggestions of behavioural differences between individuals from allopatric and sympatric populations of brown trout in this area have not been tested. In general, it is a short supply of experimental studies to clearly test the intensity of the effect of competition between the brown trout and the alpine bullhead (Vøllestad et al. 2002). The study presented in the following was for these reasons conducted to test for possible differences in behavioural responses between sympatric and allopatric populations of brown trout, exposed to alpine bullhead.

Due to ethical considerations many investigators use models instead of potential predators to study behavioural responses. Such models are however likely to lack subtle behaviours displayed by possible predators, thus altering the possible prey animal's perceived level of threat (Brown and Warburton 1999). In a situation were the exact competitor and predator role is uncertain, as in the case of brown trout and alpine bullhead, great care should be taken to ensure that all cues that could signal a threat are present.

The studies carried out so far indicate that young brown trout living in sympatry with alpine bullhead have a different activity level and positioning preference, compared to allopatric brown trout. Such behavioural responses could, among other things, explain observed differences in growth. As noted, there is little experimental confirmation to these findings. In addition, it is also not known what initiates such possible behavioural responses in activity level and positioning. Both visual and olfactory signals are known to trigger behavioural responses over a great range (Pitcher 1993). For instance, predator-sympatric rainbowfish *Melanotaenia duboulayi* Castelnau can determine the level of threat posed by a predator-fish, through visual inspection of its activity level (Brown and Warburton 1999). Prey fish also use predator odours to assess predation risk, but the level of sophistication in such risk assessments is not well known. Recent published studies indicate that odours of predators can be used by potential prey to determine the proximity of predators, as well as relative density (Ferrari et al. 2006). On the other hand, differences in activity level and positioning could be due to general characteristics of the allopatric and sympatric brown trout population, and not dependent on the presence of the sight or smell of alpine bullhead. In addition, little is known about how naive brown trout responds when exposed to stimuli of alpine bullhead. As a consequence, it is also uncertain if there would be any effect of habituation to such stimuli, with repeated exposures giving a decrease in response.

This study was designed to answer the following questions:

1. Are there behavioural differences between allopatric and sympatric brown trout, when not exposed to stimuli?
2. Are there behavioural differences between allopatric and sympatric brown trout, when exposed to sight and/or smell of alpine bullhead?
3. Are there any effects of habituation for allopatric and sympatric brown trout which experience repeated exposures?

Material and methods

The study organisms

Brown trout is a common fish throughout all of Norway, and is of great economic and recreational importance. The brown trout's colour, form and growth vary somewhat, depending on its environment. Brown trout inhabits both lotic and lentic waters. Stream dwelling brown trout lives its whole life in running water (Pethon 1998). In some areas the distribution of brown trout overlaps with that of the alpine bullhead.

Alpine bullhead can reach lengths up to 11 cm in Norway, and reach ages up to at least 6 years. In Norway, Alpine bullhead is found in Troms County, as well as in a larger area in the south eastern part of Norway. Alpine bullhead is mostly found in fast-flowing waters, often covering between, or under, stones on the bottom. Its diverse diet also contains fish eggs and larvae from brown trout (Pethon 1998).

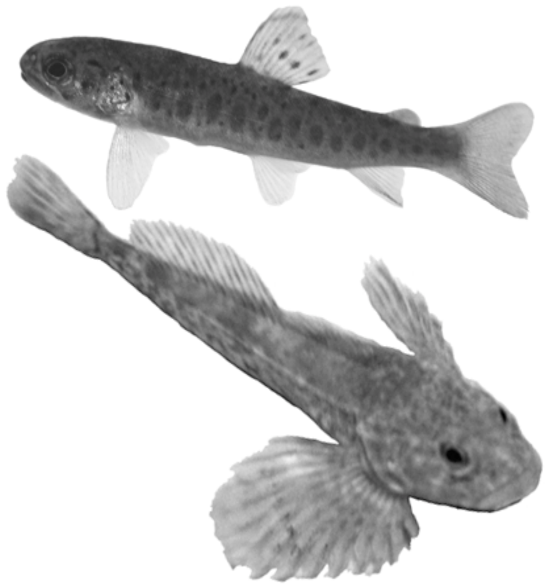


Figure 1: Brown trout and alpine bullhead from Osa, used in experiments.

The study area

The Søre Osa (hereafter Osa) river system (61° 15'N, 11° 45'E) is located in Åmot municipality, in the south-eastern part of Norway. Osa is approximately 17 km long, and runs from 436 - 235 meters above sea level (Jonsson and Sandlund 1979). The Osa river system runs through a sparsely populated area, and is surrounded by forests consisting of Scotch pine *Pinus sylvestris* and Norway spruce *Picea abies* (Jonsson and Sandlund 1979). A dam regulates the discharge into the river. The lower part of the river has a width that varies from 25-40 m, and even though the river is seldom deeper than 1 m, there are pools which can have a depth of more than 2 m. There are four main tributaries to Osa.

The river system contains brown trout, which has been isolated from the lake above (Osensjøen) by a dam since 1914 (Jonsson and Sandlund 1979). Alpine bullhead is common in the lowest part of the river, but an impassable waterfall functions as a barrier to upstream migration. An impassable waterfall also prevents migration of alpine bullhead into the Ulvåa tributary. In addition to alpine bullhead and brown trout, the Osa contains several other fish species (Jonsson and Sandlund 1979, Olsen and Vøllestad 2005). In the Ulvåa tributary there have only been reported findings of perch *Perca fluviatilis* L., which has also been reported in Osa (Olsen and Vøllestad 2005).

There have been observations of potential predators on brown trout in the area, such as mergansers *Mergus* spp., herons *Ardea cinerea* L. and mink *Mustela vison* Schreber. (Vøllestad et al. 2002).

The brown trout that was collected for this study came from two sampling sites. Brown trout and alpine bullhead were collected in a 700 m section in the lower part of Osa, near the outlet. Brown trout living in allopatry were collected several hundred meters into the Ulvåa tributary. The distance between the sampling sites at Osa and Ulvåa was less than 6 km.

The Osa is a regulated river, whereas the Ulvåa tributary is not. The bottom vegetation was quite similar on the two sites. The substratum in the lower part of Osa consists of large boulders and some areas with sand and gravel. Much of the same substratum type is found in Ulvåa, but there are considerably smaller stones, and less deep pools. Osa has been reported to have a slightly warmer temperature regime than Ulvåa (Lund et al. 2003). Earlier studies contains a more detailed view on the environmental parameters of areas nearby the sampling sites in Ulvåa and Osa (Olsen and Vøllestad 2001a).

Some downstream passage of brown trout from Ulvåa and into Osa has been reported (Olsen and Vøllestad 2001a). However, trout in these streams are resident and show no sign of extensive migration (Lund et al. 2003). In addition, there are two dams between the sampling sites, which further complicate migration.

Experimental design

Fieldwork and pre-treatment

Some brown trout was captured during early summer 2004 for testing of the setup, together with a population of alpine bullhead. Brown trout used in experiments was gathered on three occasions in August and September 2004, together with an additional population of alpine bullhead. All fish were captured using a backpack electrofishing apparatus (S. Paulsen, Trondheim, Norway). Larger trout was released, and no trout with fork length (L_F) above 8.5 cm was used in the experiments. The fish was transported by car for approximately 3 h to the University of Oslo. Here, the fish was transferred into larger holding tanks with running water (Oslo drinking water). The light period lasted 13 h and 30 min at the start of the experiments in the beginning of September, decreasing by the week towards 7 h and 15 min at the end of the experiments in early December. This was in accordance with the reported light regime at their place of origin. Both brown trout and alpine bullhead were fed defrosted red mosquito larva ("Fina Fisken", Imazo ab). Feeding took place two times a day for 5 days a week, with no feeding in weekends. Fish were fed for as long as they remained in their holding tanks. Water temperature varied with season, from maximum 11.5 °C to minimum 6.5 °C.

All the fish used in the experiments went through an acclimatization period in the holding tanks for a minimum of 7 days. The longest time in the holding tanks for the brown trout was a little under three months

Experimental setup

Four experimental aquaria were used (Akvastabil A20063). Each aquarium had a capacity of 60 L (before modification) and measured 60*30*35 cm. A glass wall was mounted inside each aquarium, dividing it into two sealed compartments as illustrated in figure 2 (see point 3). The trout-compartment measured 39.2*29.4 cm, contained water up to a height of 29.4 cm and gave room to 33.9 L of water. Water in the smaller compartment contained water up the same height as in the trout-compartment, regardless of experimental treatment. All cracks were coated with silicon, and there was no possibility for water in one compartment to leak into the other. For pictures of aquaria, see appendix 1. While the smaller compartment had still water, the trout-compartment had running water. The water entered the trout-compartment at the bottom of the aquarium near the mounted glass, through a small, fixated silicon tube. Water exited the trout-compartment via a vertical solid plastic tube with small holes at the desired surface level. This tube was mounted into a water outlet, which went through a drilled hole in the glass, near the bottom of the aquarium (see figure 2). The output of water from each brown trout compartment was measured before the start of the experiments, and after 65 % of the experiments was conducted. On both occasions there was a steady flow of exactly 1 L per minute for each aquarium.

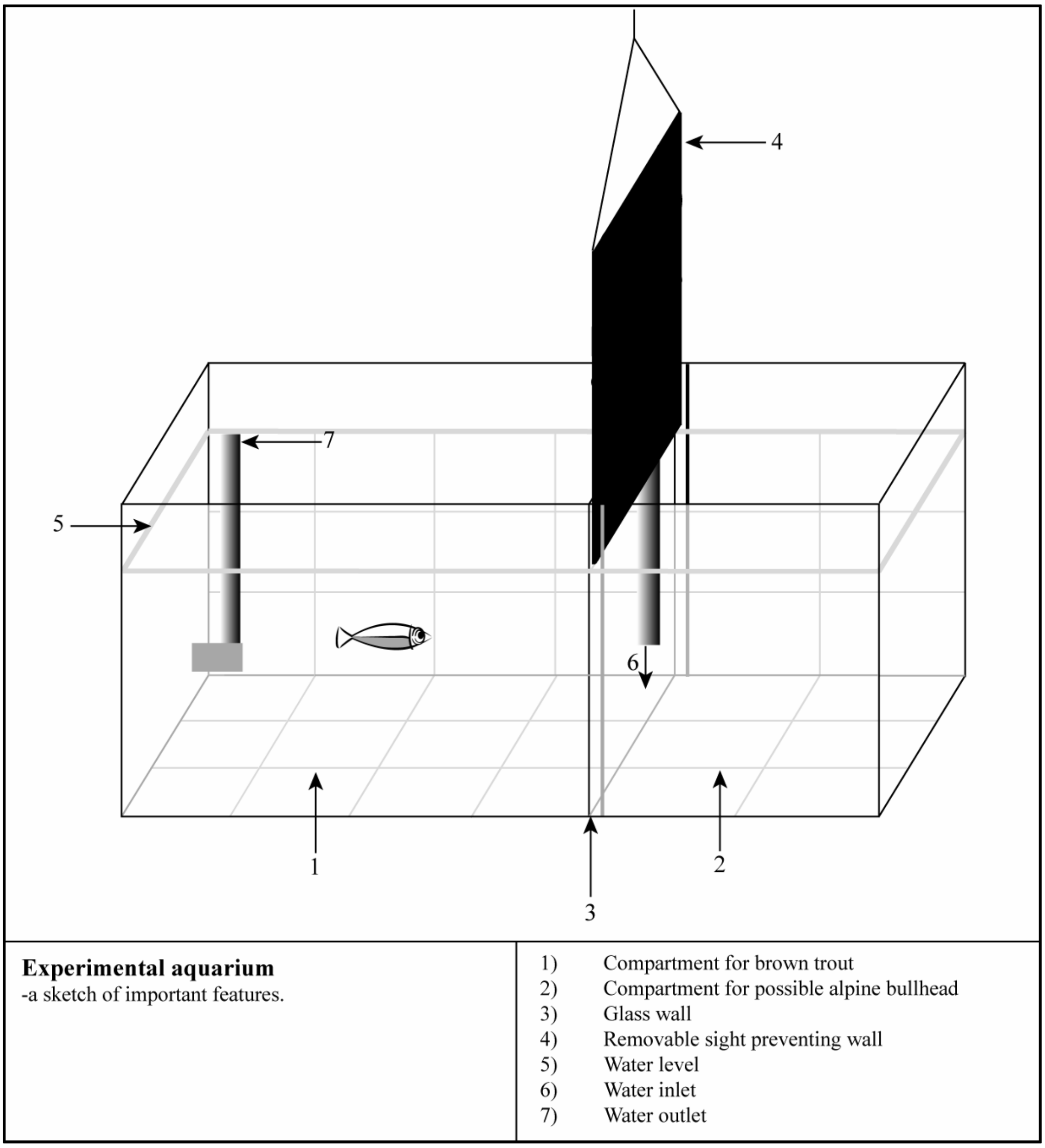


Figure 2: Features of aquaria used in all experiments

The smaller room could contain either an alpine bullhead or just water. A hanging black plastic sheet was mounted in this room, between clear rails and the glass wall dividing the two compartments, as seen in figure 2 (point 4). The sheet blocked any visual stimuli from one compartment to the other, and could be removed by the operator via a wire system.

The back and the underside of the aquaria were covered with beige cardboard containing a grid system of squares measuring 9.8*9.8 cm. This provided a grid of 3*4 squares which could be viewed through the under side, and a grid of 3*4 squares which could be viewed through the back of the aquarium. Every square was individually tagged, as seen in figure 3. The sides of the aquaria were covered with black plastic, ensuring that no fish could see fish in other experiments.

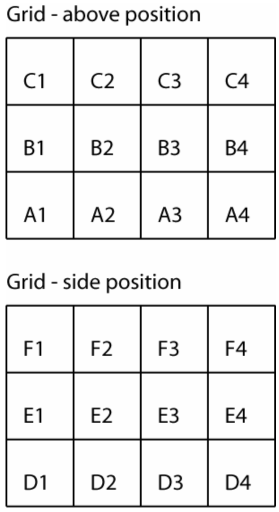


Figure 3: Design of the grid system used to measure positions and movement of brown trout in experiments

The four experimental aquaria were placed in a line on a bench, in the back of the room. 1.9 m in front of the aquaria a black plastic curtain was mounted. The curtain was extended from a beam, and it was attached to beams at both walls and at the floor. The top height of the curtain was approximately 2.2 m above the floor. The purpose of the curtain was to hide the operator from the fish used in the experiments, and to minimize disturbances. The curtain had a door which could be sealed off during experiments. Four small holes, with curtains fitting the camera lens of a digital video camera, were also made. This allowed for each aquarium to be filmed from the front. A support beam was mounted above the aquaria. The support beam and the curtain beam were equipped with rails. With the help of these rails the aquaria could be filmed from above, using a digital video camera mounted on a long rod. Four black sheets were also suspended between the beams to hide the camera rod, and to provide a shadier environment for the fish. Finally, four wire systems were mounted so the operator could stay behind the curtain, while raising the sheet blocking visual stimuli in the aquaria.

To ensure constant water pressure and water velocity of the water entering the aquaria, a modified tank was constructed. The tank was placed on a rack about 1.5 m above ground, and received water form the facility. Four water taps were mounted near the bottom of the tank, and they delivered water through flexible silicon tubes to each of the aquaria. An overflow tube was attached to the tank, allowing it to hold a constant level of above hundred litres of water at all times. The water tank had to be cleaned out at several occasions during the experiments, as it also filtered out a lot of particles in the water at the facility.

A smaller tank was mounted in the same height for the production of smell from alpine bullhead. This tank contained a shifting population of five alpine bullheads. The water was aerated with four pumps. The tank contained 100 L of still water, and was refilled if needed at the end of every day with experiments. At the end of every week the tank was washed, and the alpine bullheads replaced with other individuals. Flexible silicon tubes went from this tank, and could be fitted on the tubes leading into each aquarium. By doing this, and turning of the water taps at the ordinary water tank, it was possible to expose brown trout in any aquaria to water from the alpine bullheads in a matter of seconds. It would however take some time for this water to mix throughout the trout compartment.

In the experiments involving smell, the water entered the aquaria at the same speed as the regular water, with 1 L per minute. After 1 minute the brown trout would have received 1 L of this water, and at the end of each experiment it would have received a total of 6 L before the switch was made back to regular water. Tests were performed to check how the water spread into the aquaria. This was done by adding soluble food dye (carmine (E120) and curcumin (E100)) to the water, instead of water with the smell of alpine bullhead. With the aid of video analysis it was then possible to analyze how the water spread out in the aquaria, and the time it took to reach the different squares in the grid. The coloured water used approximately 50 seconds to cover 75% of the squares, and it took approximately two minutes to cover the entire aquaria. With these numbers in mind, it was decided to start the release of water with the smell of alpine bullhead, so that it would enter the brown trout compartment 4 minutes into the experiment, and 1 minute before the possible exposure to the sight of alpine bullhead.

Fish was not fed during their time in the experimental aquaria. Light regime was the same as for the room with holding tanks. Temperatures in the experimental aquaria varied with season from maximum 11.5 °C to minimum 6.5 °C. All aquaria were emptied and cleaned before a new brown trout was introduced to experiments.

Experimental protocol

Before the start of a set with experiments all brown trout had an acclimatization period of minimum 18 h and a maximum of 5 days in the experimental aquaria. Average time for acclimatization was 2.3 days.

Approximately one hour before the onset of a set with experiments, water was filled in the smaller compartment of the aquaria, with or without an alpine bullhead, dependent on the nature of the experiment.

The shortest amount of time between experiments on one day was approximately 2 h, and the longest time approximately 4.5 h. Average time between experiments on the same day was 3 h and 26 min. Average time for the start of experiments in the morning was 11:34 a.m. and average time for start of the experiments in the evening was 03:00 p.m. All sets of four experiments were conducted within one hour on all occasions.

All experiments were conducted in a period from 2.9.2004 to 10.12.2004. Each brown trout went through a set of four experiments over two days, as illustrated in figure 4. The same stimulus, or lack thereof, would be presented in all four experiments. This setup would allow for analyzing of any effect of habituation over time.

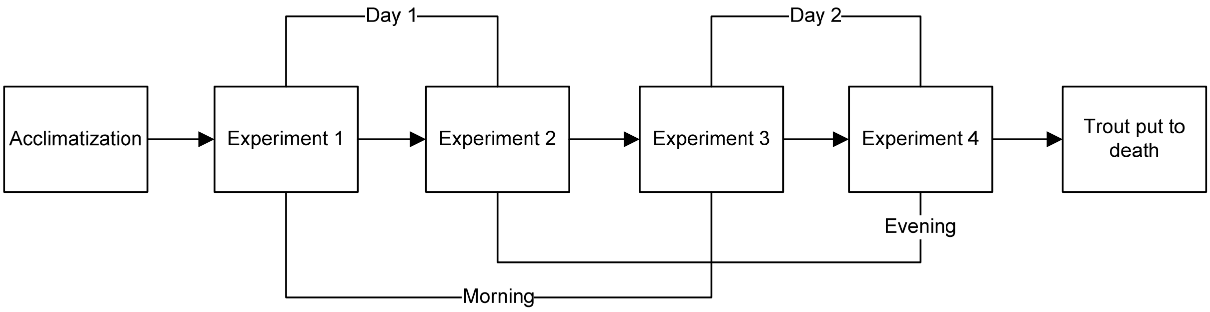


Figure 4: Flowchart depicting the experimental series over time, undergone by all fish

Every experiment lasted 10 minutes. The first four minutes the brown trout received no stimulus from alpine bullhead. The next minute the brown trout was gradually exposed to any stimulus, and the last five minutes the fish lived with the presence of any stimulus, as illustrated in figure 5. The lifting of the curtain in the small compartment would start 4:50 into the experiment and it would be fully up 5:00 into the experiment.

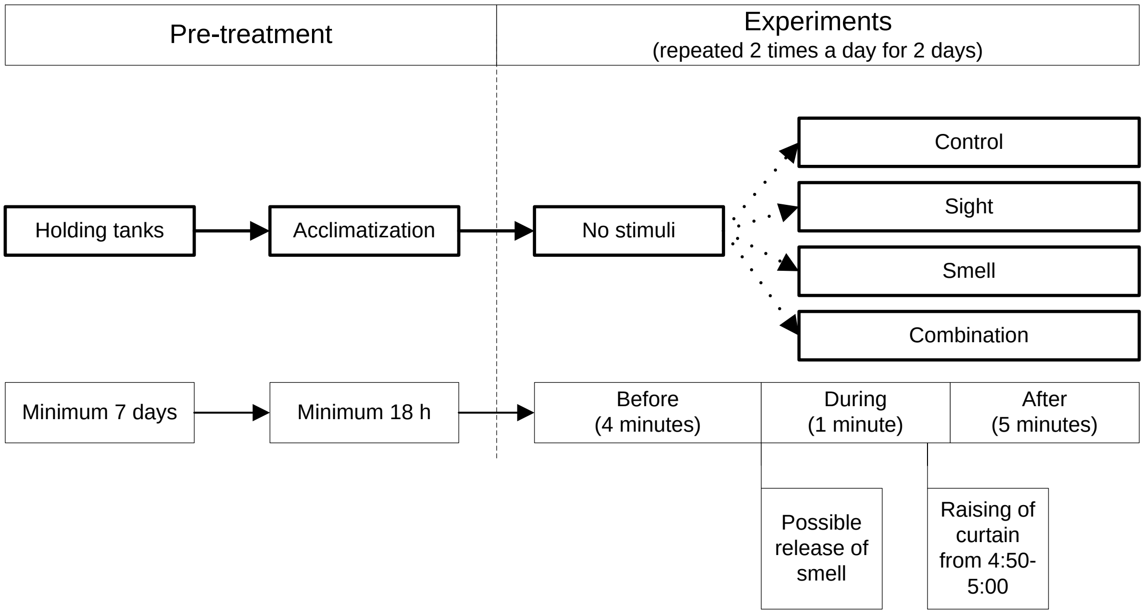


Figure 5: Flowchart depicting the four possible treatments brown trout could be subjected to throughout an experimental series

Figure 5 shows the four different types of experiments that were conducted. In the controls, the fish was treated as it would be in any of the other experiments, except that the lifting of the curtain would reveal no presence of alpine bullhead, and that the water received would remain the same. In the experiments involving only smell, the curtain would still not reveal any alpine bullhead, but the brown trout would receive water with the smell of alpine bullhead in the period 4 to 10 minutes into the experiment. In the experiments involving only sight, the water would remain the same, but the lifting of the curtain would reveal an alpine bullhead. Finally, in the experiments involving the combination of sight and smell, the brown trout would receive water with the smell of alpine bullhead in the period 4 to 10 minutes into the experiment. Five to ten minutes into the experiment it would also see an alpine bullhead.

With four aquaria it was possible to conduct four experiments at once. How many of the four brown trout that should come from the allopatric and the sympatric population was decided by drawing lots. This was done until the sympatric population was emptied. Lots were also drawn for every brown trout to decide which treatment it should receive, (control, smell, sight or the combination of smell and sight) until 25 % of the fish in each population had been used in each treatment. Finally, lots were drawn for the aquaria in which each experiment should take place. The selection of alpine bullhead for the production of smell was done at random, while alpine bullhead larger than the brown trout were selected for the experiments involving sight.

A total of 88 brown trout was used in the experiments, and each of these brown trout went through four exposures to the same treatment. As pointed out in table 1, there were two more allopatric fishes than sympatric used in the experiments. This was because one fish from the sympatric population died before experiments. This gave room for including one more allopatric fish in the experiments.

Table 1: The number of brown trout from both populations used in the different experimental treatments

	Sympatric	**Allopatric**	**All fish**
Control	11	12	**23**
Smell	10	11	**21**
Sight	11	11	**22**
Sight and smell	11	11	**22**
Total	**43**	**45**	**88**

After the brown trout had gone through four exposures in the experimental aquaria, it was exposed to a lethal dose of benzocaine. All alpine bullhead was put to death after all experiments was concluded. For all fish fork length (L_F) was measured to the nearest mm and mass to the nearest tenth of a gram. The results are presented in table 2. A t-test revealed a significant difference in mean fork length between populations (t = -4.80, df = 86, P < 0.001), with the sympatric population consisting of longer individuals than the allopatric population. I addition there was a significant difference in weight between populations (t = -5.45, df = 86, P <0.001), with generally heavier individuals in the sympatric population than in the allopatric population. From previous studies in the area, all brown trout can be assumed to be in age class 0+ or 1+ (Torgersen 2006).

Table 2: Mean fork length (± sd) and weight (± sd) for the allopatric and sympatric brown trout used in the experiments

	Fork length (cm)	**Weight (g)**
Allopatric	5.68 ± 0.51	1.62 ± 0.47
Sympatric	6.22 ± 0.56	2.22 ± 0.57

A total of 39 alpine bullheads were used during the experiments. Average fork length was 7.92 cm ± 0.94 cm and average weight was 4.93 g ± 1.71 g.

Video analysis

All experiments were filmed using two digital video cameras. One camera was filming from above, and one was filming in front of the aquaria. These positions will be referred to as the "above position" and the "side position" in the following. All film was stored on CDs. Appendix 2 gives further information on technical aspects of the video analysis.

Two types of data were recorded. To measure the activity level of the fish there were conducted counts of each time the snout of the fish crossed a line in the drawn grid of the aquaria. Since two cameras were used it was possible to take movement in all three dimensions into account. Separate counts were made for the above position and the side position and this was summed up for every minute of the 10 minute experiment. In this way all movements by all brown trout, at the level of the grid, was counted in all experiments.

To measure the preferred position of the fish during the experiments it was noted in which square the fish was placed at different time intervals. This was done separately for both cameras giving the fish place in a three-dimensional grid as a result. -5.00 was the start of the experiment, 0.00 the middle (and exposure for sight) and 5.00 was the end of the experiment. Position recording was done with the following intervals: -5.00, -4.30, -4.00, -3.30, -3.00, -2.30, -2.00, -1.30, -1.20, -1.10, -1.00, -0.50, -0.40, -0.30, -0.20, -0.10, 0.00, 0.10, 0,20, 0.30, 0,40, 0.50, 1.00, 1.10, 1.20, 1.30, 2.00, 2.30, 3.00, 3.30, 4.00, 4.30 and 5.00. Smaller steps between observations were chosen around the exposure-period, to be able to monitor this closely.

Activity level of the alpine bullhead was recorded into three categories: none, some (1-5 grid crossings) and a lot (more than 6 grid crossings). Data for population, type of experiment, exposure number, weight, fork length, which aquarium, date and time, water temperature and days before exposure in aquaria was also recorded. Together with the data from the analyses all of this was entered into an Excel-sheet (Microsoft Office Excel 2003) for statistical treatment.

Statistical methods

All statistical analyses were performed in JMP (version 5), Statistical Discovery Software, SAS Institute.

T-tests were used to test if the fork length and weight differed between allopatric and sympatric brown trout employed in experiments.

For the remaining tests only values for first exposure to treatment were used, except in the tests for possible habituation on activity level and positioning, and the test for the effect of alpine bullhead activity on activity level and positioning of brown trout.

One-way ANOVAs were used to analyze for effects of population on the activity level and positioning of brown trout before exposure.

Two-way ANOVAs were used to analyze for effects of treatment and population on changes in activity level and positioning of brown trout during experiments.

A Levene test was used to test for differences in variance between allopatric and sympatric brown trout that had been exposed to stimuli from alpine bullhead, both with respect to activity level and positioning.

Two-way ANOVA were applied to test for the effect of repeated exposures on activity level and positioning on all sympatric and allopatric brown trout. On the basis of the results, Tukey-Kramer HSD was used to test means of activity level for all brown trout with respect to exposure.

A number of factors could potentially affect the results. It is possible that brown trout was not given enough time to acclimatize in aquaria. There are studies which have found an effect of temperature on activity level of brown trout (Ojanguren et al. 2001). Further it is imaginable that the size of the brown trout can have an influence on its behaviour. In the same way, the behaviour of the alpine bullhead could be imagined to explain much of brown trout responses. Linear regressions were therefore applied to test if fork length of brown trout, temperature or number of days spent in experimental aquaria before exposure, had an effect on activity level before exposure or the change in activity level during first exposure. One-way ANOVA was used to test if the activity level of alpine bullheads in experiments had an effect on change in activity level and positioning for brown trout receiving visual stimuli.

Results

Certain patterns were already evident during observations. Most brown trout moved relatively little throughout experiments, while some individuals were consistently active. Several times brown trout was recorded with over 100 grid crossings per minute. However, most of the brown trout tended to stay near the bottom of the aquaria in square D1 – D4 (see figure 3). In the same manner there was a tendency for most brown trout to place themselves in C1, C2, B1 and B2. These positions allowed brown trout to distance themselves from the glass dividing them from possible alpine bullhead, as well as the water inlet and the areas with most turbulence in the water. It is worth mentioning that the water outlet was located in C1, and this gave some cover from above, as illustrated in figure 2, point 7.

Some brown trout seemed to have repeated visits to inspect the alpine bullhead. Other brown trout were observed to place themselves alongside the alpine bullhead, while a few individuals displayed aggressive behaviour towards the alpine bullhead. However, most brown trout seemed to have little response to the presence of alpine bullhead. The alpine bullhead did not display much aggressive behaviour towards the brown trout. On the other hand, the alpine bullhead had a much more limited space, and relatively short acclimatization periods.

The experiments resulted in 117 h and 20 min of video, and contained data on 15 416 grid crossings and 23 166 registrations of position. Statistical analyses of these data are presented in the following.

Situation before exposure

As seen in table 3, statistical treatment (one-way ANOVA) revealed no significant difference between populations in their level of activity, measured as number of grid crossings per minute. The same statistical treatment gave no significant difference between populations in positioning preference before exposure to possible stimulus from alpine bullhead. This was true for both the observations of positioning seen from the side, as well as the observations seen from the above. With regard to the behavioural aspects measured, it is clear that there were no significant differences between the behaviour of allopatric and sympatric brown trout, before being exposed to treatment.

Table 3: One-way ANOVA values for the situation before exposure with respect to the allopatric and sympatric brown trout populations (n = 88). Values are presented for activity level, position seen from the side and position seen from above.

Variable	*Sum of squares*	*F*	*P*
Activity level	95.89	1.31	0.256
Position seen for the side	0.003	1.05	0.309
Position seen from above	0.163	2.64	0.108

Appendices are included for more detail. Appendix 3 summarizes means and standard deviations for all grid crossings in all four exposures, with respect to population and experimental treatment. Appendix 4 summarizes all grid positions in a similar fashion, for both observations done from the side and observations from above.

In the remaining analysis the main focus will be on changes in activity level and positioning, rather than actual number of grid crossings and data on positions in all periods. After all, it is the changes in activity level and positioning which characterizes the possible response of brown trout to treatment.

Activity level

Table 4 sums up all changes in average number of grid crossings per minute. The value of change is derived from average number of grid crossings per minute in the periods “during” and “after” minus the period “before” (for an illustration of these periods, see figure 5). For further details on all periods, see Appendix 3.

Table 4: The mean number of grid crossings per minute during and after exposure, minus mean number of grid crossings per minute before exposure, for allopatric and sympatric brown trout used in experiments. Standard deviations are included for all values of mean. A negative mean imply lower number of grid crossings during and after exposure, than before exposure.

Treatment	Exposure 1 1. day - morning	Exposure 2 1. day - evening	Exposure 3 2. day - morning	Exposure 4 2. day - evening
	MEAN ± sd	MEAN ± sd	MEAN ± sd	MEAN ± sd
Allopatric:				
Control	-0.51 ± 4.91	1.36 ± 4.42	0.09 ± 7.70	-0.22 ± 5.59
Smell	2.83 ± 18.31	-7.39 ± 20.72	8.51 ± 13.46	-0.93 ± 17.13
Sight	2.38 ± 4.43	0.31 ± 4.85	2.95 ± 3.68	-5.17 ± 17.71
Combination	7.23 ± 4.19	3.68 ± 5.27	12.21 ± 10.87	1.42 ± 16.54
Sympatric:				
Control	1.24 ± 3.64	0.67 ± 8.88	0.86 ± 1.67	-1.14 ± 4.07
Smell	5.06 ± 6.49	2.32 ± 4.06	3.93 ± 4.33	3.18 ± 4.41
Sight	1.65 ± 3.16	0.17 ± 0.48	2.17 ± 4.85	-0.13 ± 1.72
Combination	-0.23 ± 10.11	3.39 ± 5.69	4.98 ± 7.32	3.77 ± 6.95

Table 4 reveals rather large standard deviations from the mean, supporting the observations of great individual variation in grid crossings among the brown trout. No uniform trends are apparent from the mean values.

As seen from table 5, two-way ANOVA revealed no significant difference between the brown trout populations with respect to changes in activity level. There was also no significant difference between change in activity levels with respect to treatment, including the controls. The presence of stimuli from alpine bullhead has no statistical influence on either population of alpine bullhead.

Table 5: Values from two-way ANOVA for changes in number of grid crossings during first exposure for sympatric and allopatric brown trout. Changes are measured as number of grid crossing per minute in the period under and during exposure minus grid crossing per minute in the period before exposure.

Source	*df*	*Sum of squares*	*F*	*P*
Population	1	24.21	0.34	0.559
Treatment	3	174.64	0.83	0.482
Population*Treatment	3	328.35	1.56	0.206

It was further investigated if there were consistency between populations in their variance in level of activity. No significant difference was found (F = 1.09, P = 0.300). In this analysis control-experiments were excluded, since the purpose was to investigate possible differences in the amount of variance in activity level, resulting from stimuli from the alpine bullhead.

The effect of habituation on activity level for all sympatric and allopatric brown trout was also examined. As seen in table 6, there is no effect of population, but a significant effect of exposure. However, this analysis implies a simplification, since the four exposures over time for each fish involve data which is dependant of each other. The results must therefore be read with some caution, but at least indicate that change in activity level varies with number of exposures.

Table 6: Values from a two-way ANOVA preformed to test for the effect of exposure number on the change in activity level for allopatric and sympatric brown trout populations.

Source	*df*	*Sum of squares*	*F*	*P*
Population	1	3.34	0.04	0.844
Exposure	3	1006.09	3.90	0.009
Population*Exposure	3	441.29	1.71	0.165

On basis of the significant effect of exposure, further analyses were performed. The result of a Tukey-Kramer HSD test is presented in figure 6. Mean of exposure 3 is significantly different from means for exposure 4 and 2. Exposure 1 does not have a statistically different mean from any of the other exposures. It is clear that there is no gradual decrease in activity level with time. However, the mean change in activity level is higher in the morning than in the afternoon.

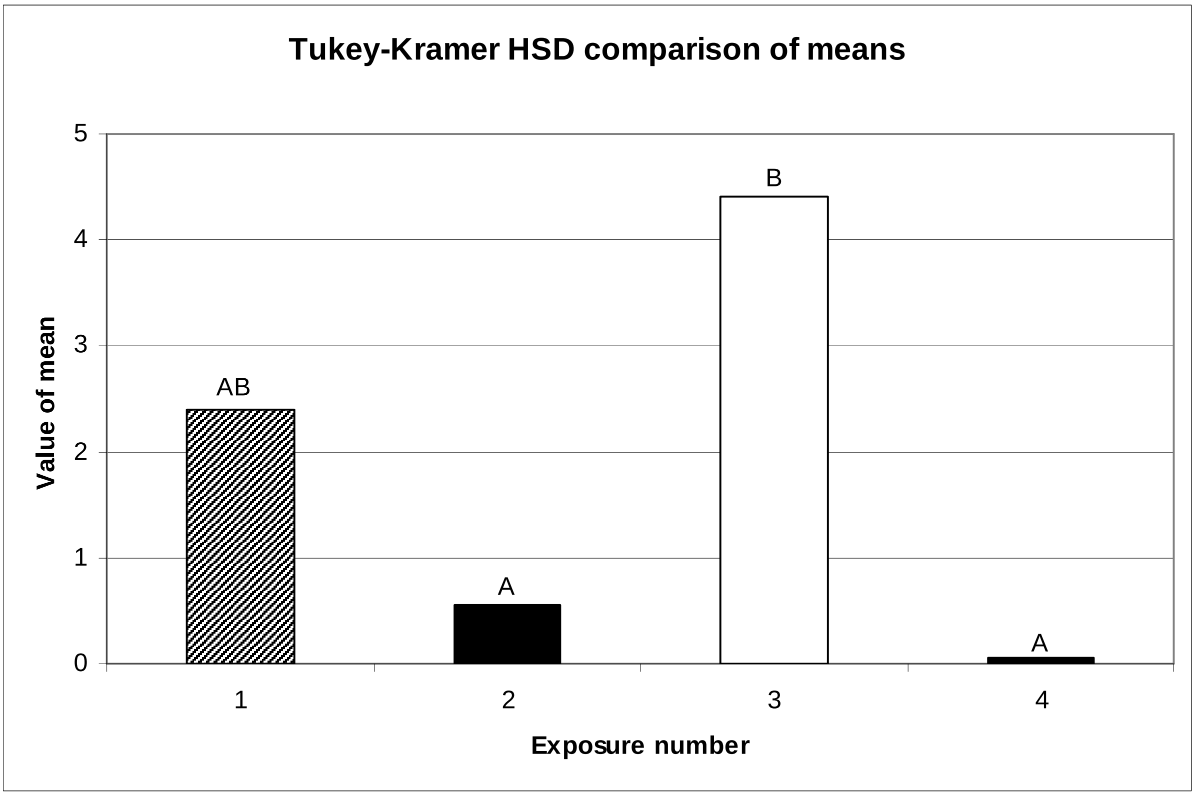


Figure 6: The result of a Tukey-Kramer HSD test for comparison of means of activity change for all brown trout in all treatments over four exposures. Change is measured as mean number of grid crossings per minute during and under exposure, minus mean number of grid crossings per minute in the period before exposure. Exposures not connected by same letter (A or B) are significantly different. Exposure 1 and 3 was conducted in the morning, while exposure 2 and 4 was conducted in the afternoon.

Positioning

Table 7 and 8 sums up the change in position for all fish involved in a treatment. The value of change is derived from average value of grid position in the period “during” + “after” – “before” (for an illustration of these periods, see figure 5). For further details on all periods, number of observations and translation of positions into numbers, see Appendix 4.

Table 7 depicts that there were very small amounts of positional changes in the height of the water column in response to treatment, for both populations of brown trout. This fits well with the general observation that most brown trout preferred to stay near the bottom of the aquaria.

Table 7: Changes in grid position for sympatric and allopatric brown trout. Mean change in grid position is estimated as mean grid position during and after exposure minus mean grid position before exposure. Values are based on data from the side position. Standard deviations are included for all values of mean. A negative value of mean imply movement deeper in the aquarium during and after exposure compared to the position before exposure.

Treatment	Exposure 1	Exposure 2	Exposure 3	Exposure 4
	1. day - morning	1. day - evening	2. day - morning	2. day - evening
	MEAN ± sd	MEAN ± sd	MEAN ± sd	MEAN ± sd
Allopatric:				
Control	-0.129 ± 0.428	-0.020 ± 0.142	-0.122 ± 0.256	-0.025 ± 0.059
Smell	0.023 ± 0.246	-0.152 ± 0.363	0.006 ± 0.299	0.003 ± 0.236
Sight	0.073 ± 0.200	-0.016 ± 0.642	0.055 ± 0.089	-0.182 ± 0.340
Combination	0.053 ± 0.090	0.020 ± 0.066	0.035 ± 0.243	0.018 ± 0.356
Sympatric:				
Control	0.008 ± 0.026	-0.019 ± 0.132	-0.009 ± 0.030	-0.023 ± 0.060
Smell	0.022 ± 0.055	0.017 ± 0.055	0.000 ± 0.000	0.017 ± 0.055
Sight	0.012 ± 0.028	0.000 ± 0.000	0.008 ± 0.018	0.000 ± 0.000
Combination	-0.022 ± 0.104	0.024 ± 0.079	0.020 ± 0.045	0.083 ± 0.208

Table 8 shows that there is more movement in the horizontal plane. Gridlines are roughly 9.8 cm apart, so a movement of one whole unit would represent near 9.8 cm of actual movement in aquaria. However, variations are considerable, as can be seen from standard deviations.

Table 8: Changes in grid position for sympatric and allopatric brown trout. Mean change in grid position is estimated as mean grid position during and after exposure minus mean grid position before exposure. Values are based on data from the above position. Standard deviations are included for all values of mean. A negative value of mean indicates movement away from the possible release of smell and the compartment for possible alpine bullhead, during and after exposure, compared to the period before exposure.

Treatment	Exposure 1	Exposure 2	Exposure 3	Exposure 4
	1. day - morning	1. day - evening	2. day - morning	2. day - evening
	MEAN ± sd	MEAN ± sd	MEAN ± sd	MEAN ± sd
Allopatric:				
Control	-0.181 ± 0.649	0.008 ± 0.357	0.105 ± 0.801	-0.007 ± 0.459
Smell	-0.189 ± 1.171	-0.096 ± 0.864	-0.255 ± 0.592	-0.001 ± 0.493
Sight	-0.170 ± 0.339	-0.105 ± 0.559	-0.548 ± 0.719	-0.127 ± 0.770
Combination	-0.282 ± 0.554	0.042 ± 0.332	-0.492 ± 0.824	-0.197 ± 0.730
Sympatric:				
Control	-0.055 ± 0.184	-0.074 ± 0.161	-0.088 ± 0.199	0.036 ± 0.124
Smell	-0.313 ± 0.863	-0.172 ± 0.691	-0.009 ± 0.506	-0.366 ± 0.961
Sight	-0.289 ± 0.626	0.034 ± 0.157	-0.017 ± 0.782	-0.099 ± 0.215
Combination	-0.061 ± 0.460	-0.111 ± 0.304	0.130 ± 0.705	-0.162 ± 1.082

Statistical analysis of the values for change in position presented in table 7, based on data from the side position, revealed a significant amount of difference between populations, as can be seen in table 9. Brown trout in the allopatric population tend to reposition themselves vertically in the water column more than brown trout from the sympatric population. The allopatric brown trout moved higher in the water column in all treatments with stimuli, while they positioned themselves deeper in the aquaria in the control-experiments. There was however no effect of treatment on either the allopatric brown trout or the more stationary sympatric brown trout.

Table 9: Two-way ANOVA for data from the side position, regarding changes in grid placement for allopatric and sympatric brown trout during first exposure. Values for change in grid placement are the result of mean grid position during and after exposure minus mean grid position before exposure.

Source	*df*	*Sum of squares*	*F*	*P*
Population	1	0.195	7.62	0.007
Treatment	3	0.026	0.34	0.794
Population*Treatment	3	0.002	0.03	0.994

Although there were more changes in positioning seen from the above position, the analysis of changes in position revealed no significant amount of difference, neither between populations or treatments, as described in table 10.

Table 10: Two-way ANOVA for data from the above position, regarding changes in grid placement for allopatric and sympatric brown trout during first exposure. Values for change in grid placement are the result of mean grid position during and after exposure minus mean grid position before exposure.

Source	*df*	*Sum of squares*	*F*	*P*
Population	1	0	0	0.996
Treatment	3	0.138	1.13	0.341
Population*Treatment	3	0.159	1.30	0.279

Taking data from the analyses of position together, analysis of the data on brown trout positioning revealed that treatment had no effect on brown trout changes in positioning. Further, there was no difference between the brown trout populations in change of position in the horizontal plane, but allopatric brown trout repositioned themselves more and on average higher in the water column, than sympatric brown trout.

The Levene test was applied to investigate whether variances in changes in position differed between the individuals from the allopatric and sympatric population. Control-experiments were excluded from this analysis. For observations done from the side position there was a significant difference in variation in positioning between populations (F: 19.64, P <0.0001). For the observations done from the above position there also was a significant amount of difference between populations (F: 7.71, P: 0.007). For observations done from side as well as from above, it is the allopatric brown trout which exhibits the most variation in their change in positioning, while sympatric trout reacts more uniformly.

The effect of habituation on change in positioning, both in the vertical and in the horizontal plane, was also examined, and the results are presented in table 11 and 12. No effect of habituation was found from the data from the side position or from the data from the above position. The statistical method used implies, as earlier noted, a simplification. Results must therefore be taken with some caution.

Table 11: Two-way ANOVA reveals no effect of habituation regarding change in position over repeated exposures for allopatric and sympatric populations of brown trout. Values are based on data from the side position. Changes in grid placement are the result of mean grid position during and after exposure minus mean grid position before exposure.

Source	*df*	*Sum of squares*	*F*	*P*
Population	1	0.020	0.42	0.517
Exposure	3	0.230	1.63	0.182
Population*Exposure	3	0.319	2.26	0.081

Table 12: Two-way ANOVA reveals no effect of habituation regarding change in position over repeated exposures for allopatric and sympatric populations of brown trout. Values are based on data from the above position. Changes in grid placement are the result of mean grid position during and after exposure minus mean grid position before exposure.

Source	*df*	*Sum of squares*	*F*	*P*
Population	1	0.091	1.94	0.164
Exposure	3	0.026	0.18	0.909
Population*Exposure	3	0.055	0.39	0.759

Confounding factors

Linear regression was used to analyze for any effect of number of days with acclimatization in aquaria, on activity level and change in activity level for all brown trout. The effect of days in aquaria on activity level before exposure was not significant (R^2 = 0.015, df = 87, P = 0.260). Neither was it found a significant effect of days in aquaria on the change in activity level during exposure (R^2 = 0.001, df = 87, P = 0.723). Data indicate that acclimatization periods have been sufficiently long.

The regression analysis depicted no significant effect of temperature on activity level before first exposure for all brown trout in all experiments, despite the fact that temperatures varied with season from maximum 11.5° C to minimum 6.5° C (R^2 = 0.001, df = 87, P = 0.739). Further, there was no effect of temperature on activity change during first exposure for all brown trout (R^2 = 0.007, df = 87, P = 0.452).

Analysis was conducted to investigate if fork length of brown trout had an effect on activity level before exposure. This was not found to be the case (R^2 = 0.002, df = 87, P = 0.682). It was also not found any significant effect of fork length on change in activity level for all brown trout (R^2 = 0.009, df = 87, P = 0.370)

The activity level of the alpine bullhead was recorded for all experiments where brown trout was subjected to treatments involving sight of the alpine bullhead. The activity level of the alpine bullhead did not affect the change in activity level for brown trout (R^2 = 0.017, df = 175, P = 0.227). Further, there was no effect of bullhead activity on position preference for brown trout observed from the side (R^2 = 0.003, df = 175, P = 0.781), or based on the data from observations from the above position (R^2 = 0.004, df = 175, P = 0.708).

Discussion

Analyses of the data made it clear that there were large individual differences in most aspects measured. In spite of these large individual differences, some trends became apparent. The results indicated no effect of stimuli from alpine bullhead on changes in activity level or positioning on either brown trout population. No effect of habituation was found, but there were a possible effect of time of day on changes in activity level for both populations. There was larger variation between allopatric brown trout when it came to changes in horizontal and vertical positioning, than between the sympatric brown trout. This was tested for all treatments which included stimuli from alpine bullhead. In all treatments allopatric brown trout changed their vertical position more than sympatric brown trout.

In the following, arguments will be presented suggesting that there is a low level of aggression and evasiveness between brown trout and alpine bullhead in Osa. This is taken to imply that interactions between brown trout in age class 0+ and 1+ and alpine bullhead, takes place on a subtle scale. Further, it will be proposed that differences in behaviour between populations could be due to different intensities of intraspecific competition.

Before exposure

Examination of the collected data indicated no difference between brown trout populations in positioning preference or in level of activity, when not exposed to stimuli from alpine bullhead. Despite a large difference in mean activity level, the individual variation was too huge to yield any significant statistical difference between populations.

Stimuli from alpine bullhead

Analysis revealed no effect of stimuli from alpine bullhead on neither position preference or activity level of the brown trout populations. The present study tested for the possibility that brown trout used olfactory cues in addition to sight to gain information from alpine bullhead. There has been many findings suggesting olfaction as a general mediator of signals involved in teleost behaviours (Pitcher 1993). Under experimental conditions brown trout has been shown to be able to detect chemical water components released by conspecifics, and to have behavioural responses to this (Ojanguren and Braña 1999). There has also been experimental studies reporting on the importance of smell to fathead minnows *Pimephales promelas* to assess proximity and number of predators (Ferrari et al. 2006). Further it has been demonstrated that the fathead minnows have the ability to make generalizations from predator odour, so that they can recognize closely related species of predators (Ferrari et al. 2007). The brown trout used in the present study was probably of a size that excluded them from possible diet of alpine bullhead (Rømme 2001). However, it is feasible that odour from alpine bullhead could contain important information leading to behavioural responses for the brown trout, had there been a high degree of aggression between species.

Brown trout from both populations did not react statistically different when exposed to stimuli from alpine bullhead, than when no stimuli were present. Taken together with the direct observations of a low level of interest from the alpine bullhead towards brown trout, it seems like there is a low level of aggression and a low tendency for avoidance between species. This assumption is further strengthened by the fact that the activity level of alpine bullhead did not influence change in position or activity level of brown trout. It is conceivable that had sympatric brown trout had much negative experience with aggression from alpine bullhead, it would have displayed a behavioural response to the level of alpine bullhead activity.

Differences between populations

An earlier experimental study of within-stream variation in early life-history traits in brown trout from this area suggested that sympatric brown trout might be more active than allopatric brown trout (Olsen and Vøllestad 2001b). Analysis of the data presented here does not find this to be the case, neither in the situation before exposure or during and after exposure.

Even though there were no effects of population on change in activity level or positioning seen from above, there was an effect of population on the change in vertical positioning in all four treatments. Allopatric brown trout changed their vertical position more than sympatric brown trout, also in the control-experiments. Most likely, this was due to a higher response to the general design of the experiments. In the control-experiments, there were no changes in the environment of the fish, except the raising of the plastic sheet as illustrated in figure 2, point 4. It is therefore likely that the allopatric brown trout responded more to this change in their environment, than did the sympatric brown trout. Direct observations, and comparisons between the sampling site in Osa and sites near the sampling site in Ulvåa, indicates that there is much higher density of brown trout in the allopatric sampling site than in the sympatric sampling site (Olsen and Vøllestad 2001a). Data presented in a diet-comparison of brown trout and alpine bullhead from Osa suggested that density of brown trout was lower in sympatry than in allopatry, due to the presence of alpine bullhead (Holmen 2001). In the present case, the difference between populations can not be explained by the presence or absence of alpine bullhead. It is possible that the higher tendency of allopatric brown trout to change their position reflects upon living with a higher degree of intraspecific competition, with higher levels of aggression among the brown trout. On the other hand, the actual changes are quite small, and care should be taken not to interpret too much from these data.

Variation in the change of activity level did not differ between populations. The allopatric brown trout varied nevertheless significantly more than the sympatric brown trout in their change in both vertical and horizontal positioning in all treatments which included stimuli from alpine bullhead. It is possible that the sympatric population had a more uniform change in positioning due to their experience with alpine bullhead. Other aquaria studies of brown trout has found wild fish to respond with less variability than hatchery fish to predator models (Berge 2002). Likewise, it has been discovered that predator sympatric rainbowfish has less variability in their responses to predators than allopatric conspecifics (Brown and Warburton 1999). However, it is hard to see how the sympatric brown trout in the present case should gain from a lower level of variation, all the time there was no significant difference between the populations in the mean change of activity level. In addition, the sympatric brown trout did not exhibit a statistical difference in their change in activity level in control experiments opposed to experiments with stimuli. As pointed out earlier, there seems to be a clear difference in brown trout densities between habitats. It is feasible that the more powerful intraspecific competition, with possible higher levels of aggression, could be reflected in more diverse strategies in positioning among the allopatric brown trout.

Sympatric brown trout was found to be significantly longer and heavier than allopatric brown trout. A previous study conducted in Osa documented that growth rate decreased with increasing density of brown trout, and that presence of alpine bullhead also had a negative influence on growth rate of brown trout, if correcting for brown trout density (Vøllestad et al. 2002). *Cottus spp*. has been shown to predate eggs and fry of brown trout, both in the wild and in laboratory studies, and in this way reduce density of brown trout populations (Gaudin and Caillere 2000, Tabor et al. 2004). The influence of stronger intraspecific competition experienced by allopatric brown trout could explain some of the differences in fork length between individuals in the allopatric and sympatric populations.

Habituation

A habituation would be characterized by a general trend in the change in activity level or positioning over the four exposures. No such effect was found for either population. Nor was it an effect of exposure on change in position. Nevertheless, a significant difference between mean changes in activity level for all brown trout was discovered between the different exposures (see figure 6). On average, there was 3 h and 26 min between exposures conducted on the same day. Figure 6 could be thought to illustrate a situation where changes in activity level were dependent on the time of day. Brown trout in the wild has been known to have activity cycles on an annual basis, as well as on a daily basis, dependent of season (Swift 1962). However, such an effect of time of day on activity level of brown trout has been attributed to differences in light intensity (Young 1999). While change in activity level during exposure 1 was several times higher than that of exposure 2 or 4, it was still not significantly different. Another behavioural study of brown trout conducted at the same facility as the experiments presented here, also found higher activity levels in the mornings than later in the day (Berge 2002). The picture remains blurry, but it is possible that the amount of change in activity level is dependent on time of day. If this is the case, this could reflect behavioural patterns varying in time, possibly related to search for nourishment or avoidance of predators.

Possible interactions

If sympatric brown trout have had much negative experience with aggression from alpine bullhead, one could hypothesize that it would react with a displacement in position when exposed to stimuli. A change in activity level could also be imagined to be caused by such aggression. Less experienced sympatric brown trout could be expected to react less uniformly due to less information on what constitutes the right level of response, as well as a lack of information when confronted with only smell of alpine bullhead. Analyses of the data presented in this study found no significant differences between populations in their change in activity level, and there were no significant differences in response for any of the treatments, including control experiments. There were however behavioural differences between the two populations. Individuals in the allopatric population repositioned themselves more vertically than the sympatric individuals. In the same way, allopatric brown trout also varied more than sympatric trout in how they repositioned themselves, both vertically and horizontally. These differences seem to reflect that allopatric trout responded more too general changes in the environment than sympatric brown trout.

Competition between the sympatric brown trout and alpine bullhead in Osa, and neighbouring streams, has been suggested in a number of earlier studies (Olsen and Vøllestad 2001b, Olsen and Vøllestad 2001a, Vøllestad et al. 2002, Holmen et al. 2003). A competitive relationship between the species has also been demonstrated in studies from other areas (Gabler and Amundsen 1999, Hesthagen and Heggenes 2003). The findings presented in this study do in no way rule out such interactions. There could also still be behavioural differences between populations with respect to stimuli from alpine bullhead. A study of the dynamics between brown trout and bullhead *Cottus gobio* L. conducted in the wild over 34 years, found the competitive relationship between species to be a dynamic process, dependent of environmental changes (Elliot 2006). As earlier studies have noted, interspecific competitive interactions may be subtle on both temporal and spatial scales (Hesthagen and Heggenes 2003). The levels that were chosen to measure differences in the present study may have been to coarse, and wrong variables may have been measured. The main methods used in this study are widely applied, with numerous variations (Brown and Warburton 1999, Berge 2002, Ferrari et al. 2007). In spite of this, behavioural responses can be quite complex, and choosing the appropriate scale for measurements can be a challenge.

It is worth noticing that several of the other studies of brown trout from this area have compared allopatric and sympatric brown trout from over and under an impassable waterfall in Osa (Holmen 2001, Olsen and Vøllestad 2001b, Holmen et al. 2003). The study presented here has used the same sympatric sampling site, but the allopatric brown trout has been caught in Ulvåa. This probably implies a larger difference in characteristics of habitats, than if both sites had been located in Osa.

There were several unsuccessful attempts during 2003 to keep brown trout from this area in captivity. Because of the general state of the facility, the possibility that not all brown trout was in good health, can not be ruled out. On the other hand, there were no more than three fish deaths in holding tanks during the period of experiments, and average time in holding tanks was one and a half month.

Taking possible objections into account, the findings presented still suggest that there is not a high degree of aggression between species, and that interactions between the species appear on a subtle scale. In addition, this study suggests that there are behavioural differences between the population in Ulvåa and Osa, not directly attributable to the presence or absence of alpine bullhead. Both streams have fairly similar habitat characteristics, beside the presence of alpine bullhead in Osa, and higher densities of brown trout in Ulvåa. Considering that smell or sight of alpine bullhead fails to explain these differences, it is here suggested that the behavioural differences between populations documented in this study could be due to different intensity of intraspecific competition within the brown trout populations. The differences in the strength of intraspecific competition could be indirectly caused by predation of alpine bullhead on brown trout fry, as well as interactions between alpine bullhead and brown trout. Further studies would be needed to investigate such a possibility.

The presented results demonstrate that studies of fish in three dimensions can reveal information on behavioural responses which otherwise would be concealed, had the fish been observed in two dimensions. The findings further demonstrate that experimental behavioural studies have the potential to play a part in broadening the understanding of ecological relationship between species. In the same way behavioural studies can generate new hypothesis about environmental constrains and ecological relationships.

Conclusion

The present study finds little evidence that competition between alpine bullhead and brown trout influence behavioural responses in sympatric brown trout, when compared to allopatric brown trout populations. Analysis of data indicates that there are behavioural differences between allopatric and sympatric brown trout, not directly attributable to presence or absence of alpine bullhead. It is here suggested that these differences is due to unequal intensities in intraspecific competition among the brown trout populations, reflecting dissimilar population densities.

References

Begon, M., Harper, J. L., and Townsend, C. R. 1996. *Ecology*. Blackwell Science, Oxford.

Berge, T. I. 2002. Anti-predator behaviour in juvenile wild and hatchery-reared brown trout (*Salmo trutta*). Pages 27. *Department of Biology*. University of Oslo, Cand. scient. thesis in zoology.

Brown, C., and Warburton, K. 1999. Differences in Timidity and Escape Responses between Predator-naive and Predator-sympatric Rainbowfish Populations. *Ethology* 105: 491-502.

Drickamer, L. C., Vessey, S. H., and Meikle, D. 1996. *Animal Behavior - mechanisms - ecology - evolution*. Wm. C. Brown Publishers.

Elliot, J. M. 2006. Periodic habitat loss alters the competitive coexistence between brown trout and bullheads in a small stream over 34 years. *Journal of Animal Ecology* 75: 54-63.

Ferrari, M. C. O., Gonzalo, A., Messier, F., and Chivers, D. P. 2007. Generalization of learned predator recognition: an experimental test and framework for future studies. *Proceedings of the Royal Society B* 274: 1853-1859.

Ferrari, M. C. O., Messier, F., and Chivers, D. P. 2006. The nose knows: minnows determine predator proximity and density through detection of predator odours. *Animal Behaviour* 72: 927-932.

Gabler, H.-M., and Amundsen, P.-A. 1999. Resource partitioning between Siberian sculpin (*Cottus poecilopus* Heckel) and Atlantic salmon parr (*Salmo salar* L.) in a sub-Arctic river, northern Norway. *Ecology of Freshwater Fish* 8: 201-208.

Gabler, H.-M., Amundsen, P.-A., and Herfindal, T. 2001. Diet segregation between introduced bullhead (*Cottus gobio* L.) and Atlantic salmon parr (*Salmo salar* L.) in a sub-Arctic river. *Archiv für Hydrobiologie* 151: 609-625.

Gaudin, P., and Caillere, L. 2000. Experimental study of the influence of presence and predation by sculpin, *Cottus gobio* L., on the drift of emergent brown trout, *Salmo trutta* L. *Archiv für Hydrobiologie* 147: 257-271.

Hesthagen, T., and Heggenes, J. 2003. Competitive habitat displacement of brown trout by Siberian sculpin: the role of size and density. *Journal of Fish Biology* 62: 222-236.

Hesthagen, T., Saksgård, R., Hegge, O., Dervo, B. K., and Skurdal, J. 2004. Niche overlap between young brown trout (*Salmo trutta*) and Siberian sculpin (*Cottus poecilopus*) in a subalpine Norwegian river. *Hydrobiologia* 521: 117-125.

Holmen, J. 2001. On competition among brown trout (*Salmo trutta* L.) and Siberian sculpin (*Cottus poecilopus* Heckel) in a Norwegian river. Pages 32. *Department of Biology*. University of Oslo, Cand. scient. thesis in zoology.

Holmen, J., Olsen, E. M., and Vøllestad, L. A. 2003. Interspecific competition between stream-dwelling brown trout and Alpine bullhead. *Journal of Fish Biology* 62: 1312-1325.

Jonsson, B., and Sandlund, O. T. 1979. Environmental factors and life histories of isolated river stocks of brown trout (*Salmo trutta* m. *fario*) in Søre Osa river system, Norway. *Environ. Biol. Fishes* 4: 43-54.

Jørgensen, L., Amundsen, P.-A., Gabler, H.-M., Halvorsen, M., Erkinaro, J., and Noemela, E. 1999. Spatial distribution of Atlantic salmon parr (*Salmo salar* L.) and bullhead (*Cottus gobio* L.) in lotic and lentic habitats of a diversified watercourse in northern Fennoscandia. *Fisheries Research* 41: 201-211.

Krebs, J. R., and Davies, N. B. 1993. *An introduction to behavioural Ecology*. Blackwell Science Ltd.

Lund, E., Olsen, E. M., and Vøllestad, L. A. 2003. First-year survival of brown trout in three Norwegian streams. *Journal of Fish Biology* 62: 323-340.

Moyle, P. B. 1977. In defense of sculpins. *Fisheries - A Bulletin of the American Fisheries Society* 2: 20-23.

Näslund, I., Degerman, E., and Nordwall, F. 1998. Brown trout (*Salmo trutta*) habitat use and life history in Swedish streams: possible effects of biotic interactions. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1034-1042.

Ojanguren, A. F., and Braña, F. 1999. Discrimination against Water Containing Unrelated Conspecifics and a Marginal Effect of Relatedness on Spacing Behaviour and Growth in juvenile Brown Trout, *Salmo Trutta* L. *Ethology* 105: 937-948.

Ojanguren, A. F., Reyes-Gavilán, F. G., and Braña, F. 2001. Thermal sensitivity of growth, food intake and activity of juvenile brown trout. *Journal of Thermal Biology* 26: 165-170.

Olsen, E. M., and Vøllestad, L. A. 2001a. Estimates of survival of stream-dwelling brown trout using mark-recaptures. *Journal of Fish Biology* 59: 1622-1637.

Olsen, E. M., and Vøllestad, L. A. 2001b. Within-stream variation in early life-history traits in brown trout. *Journal of Fish Biology* 59: 1579-1588.

Olsen, E. M., and Vøllestad, L. A. 2005. Small-scale spatial variation in age and size at maturity of stream-dwelling brown trout, *Salmo trutta*. *Ecology of Freshwater Fish* 14: 202-208.

Pethon, P. 1998. *Aschehougs store fiskebok*. Aschehoug.

Petrosky, C. E., and Waters, T. F. 1975. Annual Production by the Slimy Sculpin Population in a Small Minnesota Trout Stream. *Transactions of the American Fisheries Society* 104: 237-244.

Pihlaja, O., Julkunen, M., Niemelä, E., and Erkinaro, J. 1998. Changes in the density of introduced bullhead, *Cottus gobio* L., and its impact on juvenile Atlantic salmon, *Salmo salar* L., densities in a sub-Arctic salmon river in northern Finland. *Fisheries Management and Ecology* 5: 189-199.

Pitcher, T. J., ed. 1993. *Behaviour of Teleost Fishes*. Chapmann & Hall, London.

Rømme, H. P. 2001. Aspekter ved populasjonsdynamikk og reproduksjonsbiologi hos steinsmett (*Cottus poecilopus* Heckel). Pages 39. *Biologisk Institutt*. University of Oslo, Cand. scient.

Stearns, S. C. 1992. *The Evolution of Life Histories*. Oxford University Press.

Straskraba, M., Chiar, J., Frank, S., and Hruska, V. 1966. Contribution to the Problem of Food Competition Among the Sculpin, Minnow and Brown-Trout. *Journal of Animal Ecology* 35: 303-311.

Swift, D. R. 1962. Activity cycles in brown trout (Salmo trutta Linn.). I. Fish feeding naturally. *Hydrobiologia* 20: 241-247.

Tabor, R. A., Brown, G. S., and Luiting, V. T. 2004. The effect of Light Intensity on Sockeye Salmon Fry Migratory Bahavior and Predation by Cottids in the Cedar River, Washington. *North American Journal of Fisheries Management* 24: 128-145.

Tomaro, L. M. 2006. Apparent predation of juvenile coho salmon (*Oncorhynchus kisutch*) by prickly sculpins (*Cottus asper*) is an artefact of trapping methodology. *Marine and Freshwater Research* 57: 513-518.

Torgersen, P. 2006. Growth variation in brown trout *Salmo trutta* across small spatial scales: effects of density dependence and habitat quality? Pages 44. *Department of Biology*. Universty of Oslo, Master of Science Thesis.

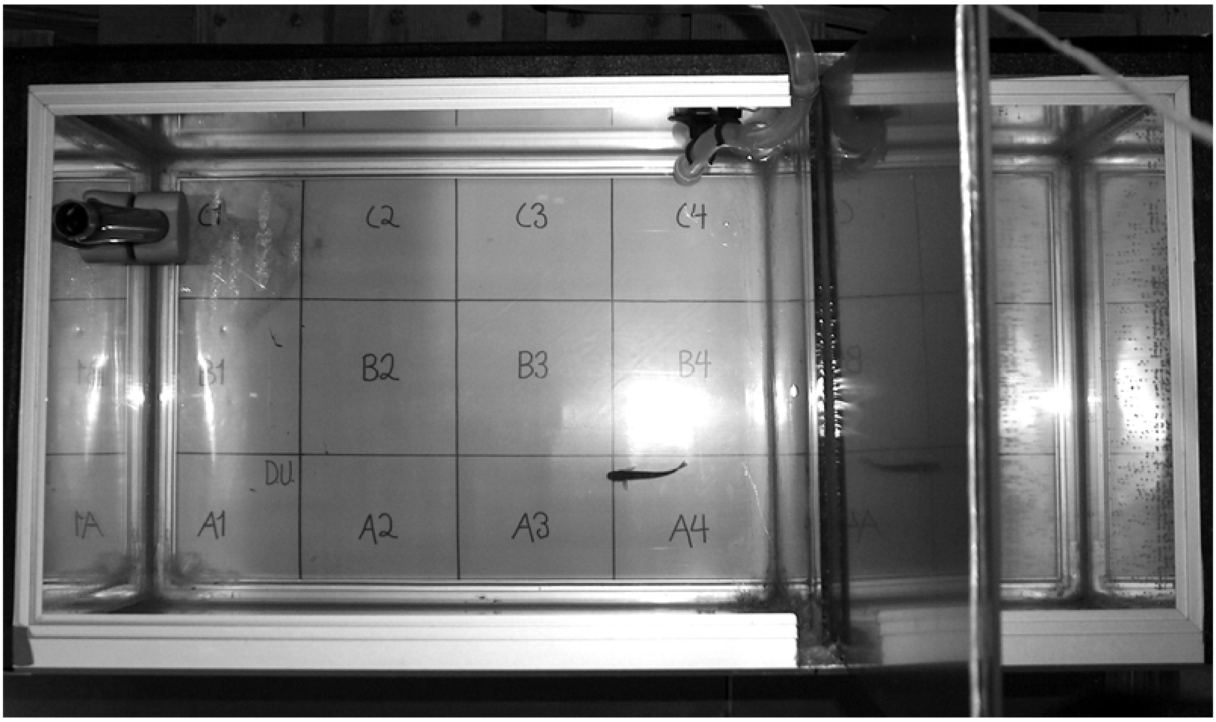
Vøllestad, L. A., Olsen, E. M., and Forseth, T. 2002. Growth-rate variation in brown trout in small neighbouring streams: evidence for density-dependence? *Journal of Fish Biology* 61: 1513-1527.

Wootton, R. J. 1998. *Ecology of Teleost Fishes*. Kluwer Academic Publishers.

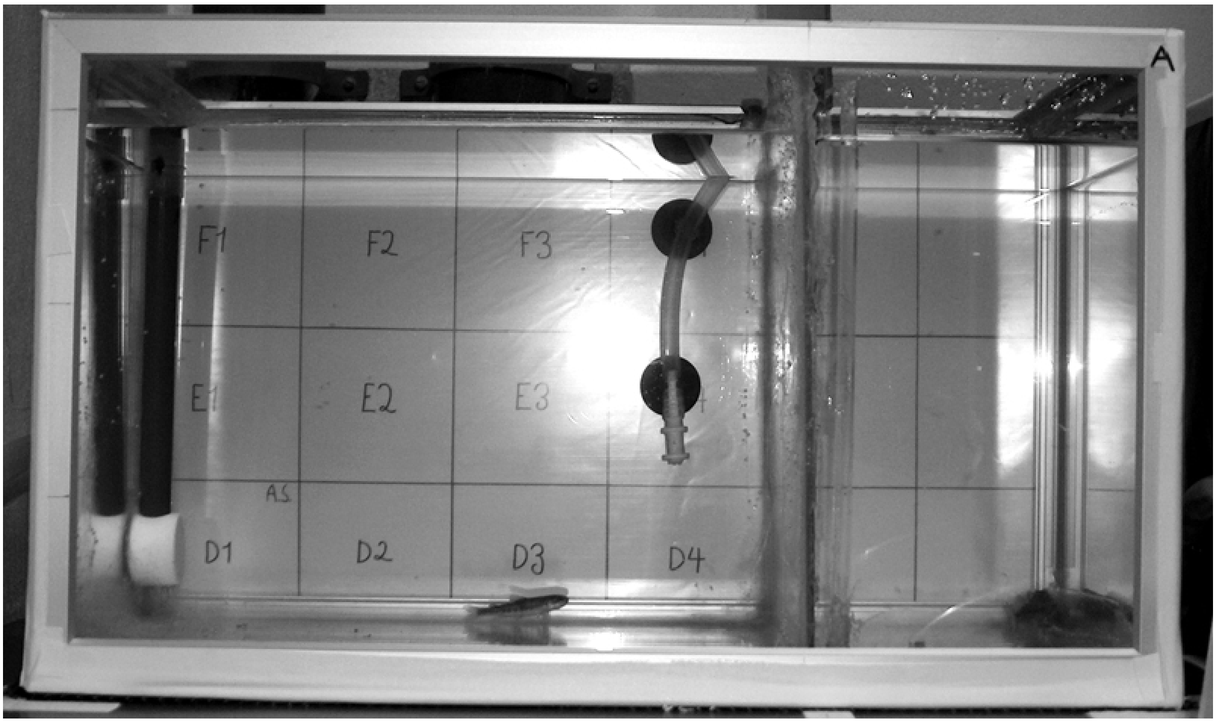
Young, M. K. 1999. Summer diel activity and movement of adult brown trout in high-elevation streams in Wyoming, U.S.A. *Journal of Fish Biology* 54: 181-189.

Appendices

Appendix 1 – Pictures of aquaria



Experimental aquarium, viewed from the above position.



Experimental aquarium, viewed from the side position.

Appendix 2 – Technical information about video analysis

The films were recorded onto DV-tapes, before they were transferred to a computer and stored on a hard disk. Capture from DV-tape, and later editing and rendering, was done with the computer program “Pinnacle Studio Plus” (version 9.3.5). After capture the experiment was numbered and start, stop and middle of the experiment was identified by sound signals. In some of the videos contrast and lighting was manipulated digitally, to make up for low lightning in experiments.

Since digital video requires a massive amount of storage capacity, the resolution of the film was altered before rendering and storage onto CD-ROM. The resolution was altered according to the standard referred to as Video-CD (Codec: MPEG-1, resolution 352*288 and 25 frames per second). Although this lowered the quality of the films, it was deemed as more than sufficient for the needs of the analysis.

All CDs with film were labelled and delivered to an independent third-person, (Mr. Thomas Brevig at the IT-department, IMBV) for renaming of the discs. This was done to ensure an unbiased analysis of the material. Even though the analyzer would be able to see the presence of alpine bullhead, it would be impossible to detect if smell was involved, or from which population the brown trout originated. After all analyzes were conducted, the labelling was decoded.

The sum of all experiments gave a total of 117 h and 20 min of film, which was analyzed. The program used for viewing and analyzing the films was InterVideo WinDVD (version 4.0).

The use of digital film on a computer allowed for very accurate measurements. Film could be stopped and slowed down in a very precise manner, and details could be enhanced if necessary.

Appendix 3 - Summary of values for grid crossings per minute for all brown trout

Before: -5:00 - -1:00
During: -1:00 - -0:00
After: 0:00 - 5:00
All exposure: -1:00 - 5:00

Mean: average number of grid crossings per minute for all fish in a treatment
sd: standard deviation
Change Mean: Mean all exposure - mean before

Treatment	Exposure 1									
	Before		During		After		All exp.		Change	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd
Allopatric:										
Control	3.500	12.124	3.583	8.533	2.867	7.830	2.986	7.778	-0.514	4.912
Smell	5.705	15.394	12.091	12.202	7.818	11.160	8.530	9.749	2.826	18.315
Sight	1.682	3.888	4.636	11.801	3.945	9.496	4.061	8.256	2.379	4.432
Combination	2.000	4.562	10.182	13.333	9.036	6.585	9.227	6.878	7.227	4.187
Sympatric:										
Control	0.000	0.000	0.000	0.000	1.491	4.368	1.242	3.640	1.242	3.640
Smell	0.075	0.237	1.900	3.479	5.780	7.722	5.133	6.422	5.058	6.493
Sight	0.000	0.000	0.909	2.071	1.800	3.774	1.652	3.158	1.652	3.158
Combination	4.386	13.022	1.455	4.503	4.691	5.928	4.152	5.398	-0.235	10.106

Treatment	Exposure 2									
	Before		During		After		All exp.		Change	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd
Allopatric:										
Control	1.208	3.882	3.500	6.987	2.383	3.547	2.569	3.587	1.361	4.424
Smell	20.477	30.627	19.364	23.897	11.836	19.332	13.091	17.607	-7.386	20.718
Sight	1.705	4.548	2.818	3.545	1.855	1.676	2.015	1.473	0.311	4.847
Combination	0.227	0.754	5.455	10.875	3.600	6.325	3.909	5.955	3.682	5.274
Sympatric:										
Control	3.432	7.136	1.091	3.015	4.709	10.379	4.106	9.078	0.674	8.884
Smell	0.600	1.729	0.000	0.000	3.500	5.652	2.917	4.710	2.317	4.056
Sight	0.045	0.151	0.000	0.000	0.255	0.515	0.212	0.429	0.167	0.477
Combination	0.000	0.000	0.000	0.000	4.073	6.832	3.394	5.694	3.394	5.694

Treatment	Exposure 3									
	Before		**During**		**After**		**All exp.**		**Change**	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd
Allopatric:										
Control	5.229	12.310	8.333	12.521	4.717	9.941	5.319	10.103	0.090	7.696
Smell	5.295	9.343	6.727	9.645	15.218	16.763	13.803	14.677	8.508	13.463
Sight	0.659	1.236	9.364	16.645	2.455	3.071	3.606	3.606	2.947	3.683
Combination	1.500	3.970	5.636	8.334	15.327	12.759	13.712	10.459	12.212	10.874
Sympatric:										
Control	0.477	1.502	1.000	1.483	1.400	2.467	1.333	2.251	0.856	1.665
Smell	0.000	0.000	1.900	3.107	4.340	5.281	3.933	4.328	3.933	4.328
Sight	0.727	1.679	1.818	2.639	3.109	5.694	2.894	4.731	2.167	4.851
Combination	0.000	0.000	2.091	6.008	5.564	8.548	4.985	7.321	4.985	7.321

Treatment	Exposure 4									
	Before		**During**		**After**		**All exp.**		**Change**	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd
Allopatric:										
Control	3.583	11.330	4.583	9.405	3.117	5.712	3.361	6.201	-0.222	5.592
Smell	9.477	18.997	7.909	17.410	8.673	12.979	8.545	12.691	-0.932	17.128
Sight	14.977	30.381	22.273	35.972	7.309	9.968	9.803	13.388	-5.174	17.707
Combination	5.000	16.254	2.727	7.115	7.164	9.097	6.424	7.500	1.424	16.536
Sympatric:										
Control	10.023	23.072	11.364	26.741	8.382	21.314	8.879	22.038	-1.144	4.074
Smell	0.150	0.474	1.000	1.764	3.800	5.058	3.333	4.256	3.183	4.411
Sight	0.932	2.133	1.545	3.236	0.655	1.976	0.803	1.996	-0.129	1.724
Combination	0.295	0.828	3.273	7.617	4.218	8.463	4.061	6.996	3.765	6.953

Appendix 4 - Summary of values for grid positions for all brown trout

Before	-5:00 - -1:00	(10 observations)
During:	-1:00 - -0:00	(6 observations)
After:	0:00 - 5:00	(17 observations)
All exposure:	-1:00 - 5:00	(23 observations)

Mean:	average grid position for all fish involved in a treatment
sd:	standard deviation
Change Mean:	Mean all exposure - mean before

Above

Categories	**Value**
Fish in grid A1, B1 and C1:	1
Fish in grid A2, B2 and C2:	2
Fish in grid A3, B3 and C3:	3
Fish in grid A4, B4 and C4:	4

Side

Categories	**Value**
Fish in grid D1, D2, D3 and D4:	1
Fish in grid E1, E2, E3 and E4:	2
Fish in grid F1, F2, F3 and F4:	3

Figure 3 in "Material and methods" give a visual illustration of the grid system.

<u>Above:</u>

Treatment	**Exposure 1**									
	Before		**During**		**After**		**All exp.**		**Change**	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd
Allopatric:										
Control	2.358	1.277	2.361	1.275	2.113	1.216	2.178	1.170	-0.181	0.649
Smell	2.264	1.295	2.288	1.183	2.000	0.906	2.075	0.842	-0.189	1.171
Sight	2.845	1.154	2.894	1.151	2.599	1.066	2.676	1.066	-0.170	0.339
Combination	2.282	1.256	2.288	1.247	1.898	0.886	2.000	0.940	-0.282	0.554
Sympatric:										
Control	1.727	1.191	1.727	1.191	1.652	1.051	1.672	1.084	-0.055	0.184
Smell	1.830	1.174	1.650	0.944	1.471	0.664	1.517	0.656	-0.313	0.863
Sight	2.455	0.934	2.455	0.934	2.064	0.829	2.166	0.773	-0.289	0.626
Combination	2.045	1.294	2.000	1.265	1.979	1.085	1.984	1.095	-0.061	0.460

Treatment	**Exposure 2**									
	Before		**During**		**After**		**All exp.**		**Change**	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd
Allopatric:										
Control	2.242	1.290	2.250	1.278	2.250	1.163	2.250	1.174	0.008	0.357
Smell	2.527	1.202	2.273	1.254	2.487	1.218	2.431	1.150	-0.096	0.864
Sight	2.591	1.319	2.636	1.362	2.433	1.125	2.486	1.150	-0.105	0.559
Combination	1.345	0.658	1.273	0.647	1.428	0.576	1.387	0.577	0.042	0.332
Sympatric:										
Control	1.809	1.134	1.818	1.168	1.706	0.943	1.735	1.000	-0.074	0.161
Smell	2.050	1.012	2.100	0.994	1.800	0.779	1.878	0.744	-0.172	0.691
Sight	2.409	1.393	2.455	1.368	2.439	1.353	2.443	1.357	0.034	0.157
Combination	2.091	1.300	2.091	1.300	1.941	1.010	1.980	1.078	-0.111	0.304

Treatment	**Exposure 3**									
	Before		**During**		**After**		**All exp.**		**Change**	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd
Allopatric:										
Control	2.025	0.857	2.236	0.922	2.093	1.258	2.130	1.132	0.105	0.801
Smell	2.836	1.270	2.773	1.252	2.513	1.213	2.581	1.197	-0.255	0.592
Sight	2.836	1.255	2.636	1.171	2.166	0.996	2.289	1.007	-0.548	0.719
Combination	2.382	1.366	2.455	1.440	1.690	0.632	1.889	0.759	-0.492	0.824
Sympatric:										
Control	1.764	1.027	1.727	1.009	1.658	1.002	1.676	1.001	-0.088	0.199
Smell	2.100	0.994	2.167	1.080	2.065	0.882	2.091	0.907	-0.009	0.506
Sight	2.555	1.194	2.667	1.254	2.492	1.299	2.538	1.192	-0.017	0.782
Combination	1.636	0.924	1.606	0.917	1.824	0.989	1.767	0.882	0.130	0.705

Treatment	**Exposure 4**									
	Before		**During**		**After**		**All exp.**		**Change**	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd
Allopatric:										
Control	2.192	1.208	2.292	1.287	2.147	0.934	2.185	1.006	-0.007	0.459
Smell	2.555	1.296	2.500	1.267	2.572	1.310	2.553	1.288	-0.001	0.493
Sight	2.609	1.092	2.803	1.087	2.369	1.008	2.482	0.937	-0.127	0.770
Combination	2.209	1.449	2.197	1.458	1.947	1.158	2.012	1.162	-0.197	0.730
Sympatric:										
Control	1.300	0.427	1.394	0.664	1.316	0.462	1.336	0.468	0.036	0.124
Smell	2.170	1.358	1.883	1.149	1.776	1.006	1.804	1.032	-0.366	0.961
Sight	2.909	1.158	2.818	1.168	2.807	1.218	2.810	1.197	-0.099	0.215
Combination	2.000	1.265	1.833	1.083	1.840	1.017	1.838	0.912	-0.162	1.082

Side:

Treatment	Exposure 1									
	Before		**During**		**After**		**All exp.**		**Change**	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd
Allopatric:										
Control	1.242	0.611	1.264	0.641	1.059	0.170	1.112	0.248	-0.129	0.428
Smell	1.100	0.224	1.227	0.436	1.086	0.213	1.123	0.211	0.023	0.246
Sight	1.018	0.060	1.030	0.101	1.112	0.353	1.091	0.260	0.073	0.200
Combination	1.018	0.060	1.106	0.261	1.059	0.105	1.071	0.102	0.053	0.090
Sympatric:										
Control	1.000	0.000	1.000	0.000	1.011	0.035	1.008	0.026	0.008	0.026
Smell	1.000	0.000	1.000	0.000	1.029	0.075	1.022	0.055	0.022	0.055
Sight	1.000	0.000	1.000	0.000	1.016	0.038	1.012	0.028	0.012	0.028
Combination	1.045	0.151	1.015	0.050	1.027	0.061	1.024	0.056	-0.022	0.104

Treatment	Exposure 2									
	Before		**During**		**After**		**All exp.**		**Change**	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	Sd
Allopatric:										
Control	1.183	0.575	1.222	0.574	1.142	0.403	1.163	0.445	-0.020	0.142
Smell	1.318	0.417	1.212	0.342	1.150	0.240	1.166	0.199	-0.152	0.363
Sight	1.336	0.751	1.364	0.809	1.305	0.626	1.320	0.554	-0.016	0.642
Combination	1.000	0.000	1.030	0.101	1.016	0.053	1.020	0.066	0.020	0.066
Sympatric:										
Control	1.055	0.129	1.000	0.000	1.048	0.126	1.036	0.093	-0.019	0.132
Smell	1.000	0.000	1.000	0.000	1.024	0.074	1.017	0.055	0.017	0.055
Sight	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
Combination	1.000	0.000	1.000	0.000	1.032	0.106	1.024	0.079	0.024	0.079

Treatment	Exposure 3									
	Before		**During**		**After**		**All exp.**		**Change**	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd
Allopatric:										
Control	1.383	0.746	1.292	0.660	1.250	0.510	1.261	0.548	-0.122	0.256
Smell	1.136	0.269	1.076	0.202	1.166	0.191	1.142	0.159	0.006	0.299
Sight	1.182	0.603	1.288	0.597	1.219	0.594	1.237	0.591	0.055	0.089
Combination	1.064	0.211	1.030	0.101	1.123	0.211	1.099	0.158	0.035	0.243
Sympatric:										
Control	1.009	0.030	1.000	0.000	1.000	0.000	1.000	0.000	-0.009	0.030
Smell	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
Sight	1.000	0.000	1.000	0.000	1.011	0.024	1.008	0.018	0.008	0.018
Combination	1.000	0.000	1.000	0.000	1.027	0.061	1.020	0.045	0.020	0.045

Treatment	Exposure 4									
	Before		**During**		**After**		**All exp.**		**Change**	
	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd	MEAN	sd
Allopatric:										
Control	1.217	0.587	1.278	0.664	1.162	0.507	1.192	0.533	-0.025	0.059
Smell	1.127	0.283	1.121	0.270	1.134	0.211	1.130	0.177	0.003	0.236
Sight	1.700	0.852	1.833	0.963	1.406	0.581	1.518	0.634	-0.182	0.340
Combination	1.073	0.241	1.015	0.050	1.118	0.336	1.091	0.247	0.018	0.356
Sympatric:										
Control	1.118	0.271	1.167	0.373	1.070	0.195	1.095	0.232	-0.023	0.060
Smell	1.000	0.000	1.000	0.000	1.024	0.074	1.017	0.055	0.017	0.055
Sight	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
Combination	1.000	0.000	1.000	0.000	1.112	0.281	1.083	0.208	0.083	0.208