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Challenging fear: chemical alarm signals are not causing morphology changes in crucian carp (*Carassius carassius*)

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Abstract Crucian carp develops a deep body in the presence of chemical cues from predators, which makes the fish less vulnerable to gape-limited predators. The active components originate in conspecifics eaten by predators, and are found in the filtrate of homogenised conspecific skin. Chemical alarm signals, causing fright reactions, have been the suspected inducers of such morphological changes. We improved the extraction procedure of alarm signals by collecting the supernatant after centrifugation of skin homogenates. This removes the minute particles that normally make a filtered sample get turbid. Supernatants were subsequently diluted and frozen into ice-cubes. Presence of alarm signals was confirmed by presenting thawed ice-cubes to crucian carp in behaviour tests at start of laboratory growth experiments. Frozen extracts were added further on three times a week. Altogether, we tested potential body-depth-promoting properties of alarm signals twice in the laboratory and once in the field. Each experiment lasted for a minimum of 50 days. Despite growth of crucian carp in all experiments, no morphology changes were obtained. Accordingly, we conclude that the classical alarm signals that are releasing instant fright reactions are not inducing morphological changes in this species. The chemical signals inducing a body-depth increase are suspected to be present in the particles removed during centrifugation (i.e., in the precipitate). Tissue particles may be metabolized by bacteria in the intestine of predators, resulting in water-soluble cues. Such latent chemical signals have been found in other aquatic organisms, but hitherto not reported in fishes.

Keywords Inducible defences · Body depth · Olfaction · Growth · Goldfish · Schreckstoff

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Introduction

Inducible defences caused by chemical cues are widespread among aquatic organisms (Harvell 1990; Tollrian and Harvell 1999). Crucian carp (*Carassius carassius*) is known to develop a deep body in the presence of chemical cues from predators, like northern pike, *Esox*

lucius, and perch, *Perca fluviatilis* (Brönmark and Miner 1992; Brönmark and Pettersson 1994). This change in morphology is detectable after 40–50 days. Deeper bodies make the prey fish less vulnerable to gape-limited predators, and also enhance the escape locomotor performance of prey (Nilsson et al. 1995; Nilsson and Brönmark 2000; Domenici et al. 2008).

Following the discovery of predator induced body depth increase in crucian carp, classical alarm signals were suspected to be the active inducers (Brönmark and Miner 1992; Brönmark and Pettersson 1994). Such chemical signals ("Schreckstoff") are present in epidermal club cells of cyprinid fish (i.e. alarm substance cells—ASC) and induces fright behaviour in conspecifics when released into the environment upon injury (von Frisch 1938, 1941; Pfeiffer 1960). In growth experiments carried out by Brönmark and Pettersson (1994), chemical cues released by perch that had eaten crucian carp, and not perch that had eaten macro-invertebrates (chironomids), effected body morphology of crucian carp. However, simulated predator attacks with injured conspecifics three times a week, carried out by immersing a crucian carp in the experimental aquarium and scratching its sides with a knife, failed to induce such a body depth increase. Accordingly, the morphological change was ascribed to the piscivorous diet of the predator, and it was concluded that the chemical substance causing a change originated in the predator (Brönmark and Pettersson 1994).

It was later reported that exposure to a pike predator with a diet of Arctic char (*Salvelinus alpinus*), a species without club cells (Pfeiffer 1977), was not a sufficient cue to induce a body depth increase in crucian carp while there was an effect by the same predator when crucian carp had been eaten (Stabell and Lwin 1997). Because the active components were also found in filtered, homogenized skin of crucian carp, the chemical signals inducing a phenotypic change were proposed similar to those triggering behavioural alarm effects, i.e. by the classical alarm signals (von Frisch 1941). Such signals may be released by predators that have been labelled with chemical cues from crucian carp following ingestion of prey (e.g., Mathis and Smith 1993a, b; Pettersson et al. 2000). However, Stabell and Lwin (1997) did not exclude the possibility that the chemical signals inducing a phenotypic change and the signals triggering an alarm response represented different sets of chemical compounds present in epidermal cells of crucian carp. An increase in body depth has been found also in goldfish (*Carassius auratus*), a close relative to crucian carp, resulting from exposure to predators fed goldfish as well as filtered skin homogenates of that species (Chivers et al. 2008). Even other fish species, like perch and roach (*Rutilus rutilus*), have been found to undergo phenotypic changes when exposed to chemical cues from pike predators (Eklöv and Jonsson 2007).

We wanted to further explore the mechanisms underlying body depth increase in crucian carp following exposure to conspecific skin extracts. In particular, we wanted to examine if the classical alarm signals (e.g., von Frisch 1941) are the causative agents of this phenotypic change. We therefore improved the procedure for producing extracts of the water-soluble alarm signals, using centrifugation instead of filtering of skin homogenates. However, in the subsequent experiment no body-depth increase was found in alarm-signal exposed fish compared to similar skin extracts obtained from brown trout (*Salmo trutta*). This led us to explore if the choice of non-cyprinid skin for control extract are involving any possible sources of error, and if crucian carp respond with morphological changes when exposed to chemical alarm signals in the field. Accordingly, we carried out two additional experimental series with improved skin extracts. The unequivocal results obtained from all three experiments are reported here.

Material and methods

General procedures

We used crucian carp as the species of study in three series of experiments, carried out in Norway and Estonia. In most cases, we captured fish by traps made from large polycarbonate water bottles (18.9 L) using canned corn as bait, followed by transportation of the catch to the local experimental facility. Seining was used as a supplementary method in one pond in Estonia. In all three experimental series, only fish less than 10 cm in length were used. All handling, transport and rearing was carried out in accordance with the respective national legislations for treatment of animals (The Norwegian Animal Welfare Act of

1974, and the Regulation of Animal Experimentation of 1998, chapter III; The Estonian Law of Animal Protection, RTI - 09.01.2001).

We selected fish by size for growth experiments, followed by anaesthetizing, weighing to the nearest 0.1 g, and photographing with a digital camera against a background millimetre grid before distributing them to rearing or transport tanks. Photos and weight were later used for morphometric analysis. For skin extracts, we sacrificed supplementary fish by a blow to the head and stored them at −20°C until use.

Preparation of skin extracts

The procedure for preparing skin extract of fishes was identical for all experiments reported, deviating slightly from standard procedure (e.g., Stabell and Lwin 1997). Fishes were lightly thawed, and incisions were made behind the gill and along the dorsal and ventral edges on each side, and the skin was peeled off using forceps. With this procedure we obtain a skin layer devoid of flesh. The skin was then homogenised in a blender with 100–150 ml of tap water, the homogenate was centrifuged at 2500 rpm for 5 min, and the supernatant was subsequently collected and diluted with tap water to a final volume of 3.15 l. The diluted extracts were then transferred to ice-cube bags (350 ml each) and frozen at −20°C. Freezing has previously not been found to affect the functional properties of chemical alarm signals in fishes (Lawrence and Smith 1989; Mathis and Smith 1993a, b; Stabell and Lwin 1997).

Centrifugation of homogenates to obtain a particle-free supernatant, as used in this study, deviates from previously reported procedures where filtering have been used (Pfeiffer 1962; Stabell and Lwin 1997; Chivers et al. 2008). Accordingly, we introduced thawed samples at start of the laboratory experiments to confirm the presence of alarm signals in the diluted and frozen supernatants (Experiment 1 and 2). This first-time introduction of stimuli was accompanied by video monitoring.

Experiment 1—laboratory

Experimental fish and exposure types

We captured crucian carp in the Springvannsdammen pond, located within the city borders of Arendal, Agder County, Norway (58°31′N; 8°46′E). The pond has a surface area of 0.08 ha, with crucian carp as the only fish species. Captured fish were transported to the Department of Natural Sciences, University of Agder. Following anaesthetization (chlorbutanol – 30 mg l^{-1}), the fish were randomly distributed to their experimental aquaria.

Brown trout were brought live to the laboratory from the Syrtveit Hatchery at Evje in the Setesdal Valley, for subsequent use as donors for control skin extracts. Salmonids are not possessing epidermal club cells, and are not displaying behavioural fright reaction resulting from exposure to conspecific skin extracts (Pfeiffer 1977). The trout were sacrificed by a blow to the head, and frozen at −20°C until use. We made skin extracts from 2.8 g of skin of each species, obtained from two crucian carps and five brown trout.

Experimental design and rearing

In the rearing experiments, we raised altogether 48 crucian carp in groups of four for 50 days at 18–20°C on a 16 L : 8D photoperiod. Rearing was done in 20 l aquaria, containing water treated with 0.5 ml l^{-1} Aquasafe (Tetra GmbH, Melle, Germany) and aerated by air-stones. We used triplicate aquaria within four exposure series in a randomized block design.

Within each block, we added conspecific skin extract as frozen ice cubes (approx. 37.5 ml·aquarium^{-1}) three times a week in two aquaria, while a similar amount of frozen skin extract of brown trout was added in the other two aquaria. For both exposure types, we provided the fish either a low (0.03 g/fish/day) or a high (0.1 g/fish/day) feed ration per day. A mixture of commercial fish feed (Tetra AniMin Goldfish Colour, Tetra GmbH, Melle, Germany) and rolled oats in the ratio 1:1 by weight was used, representing approx. 1% and 3% of average fish biomass per day.

Experiment 2—laboratory

Experimental fish, skin extracts and rearing

Also in this experiment, we brought crucian carp from the Springvannsdammen pond to the Department of Natural Sciences, followed by anaesthetizing (benzocaine—50 mg l^{-1}) and random distribution to their experimental aquaria. We used triplicate aquaria with

four fish in each for an exposure series, and four exposure series were run in parallel in a randomized block design. Rearing conditions were the same as used for Exp.1, and the fish were given a feed ration of approx. 0.05 g/fish/day over a period of 50 days.

The four stimuli types were skin extracts of crucian carp, brown trout, and Arctic char, plus tap water as blank treatment. Brown trout was again obtained from the Syrtveit Hatchery, while Arctic char were brought live by air from Kårvik Research Station outside Tromsø, North Norway. Extracts were made from skin of three crucian carp (2.7 g), five brown trout (3.4 g), and three Arctic chars (3.6 g).

Experiment 3—field

Experimental fish and stimuli

We captured small, shallow bodied crucian carp in two distant, predator free ponds, Laeva and Vellavere, in the Lake Võrtsjärv area south-west of Tartu, Estonia. The Laeva pond measures 0.11 ha, with a water depth of 1.5 m, and contain goldfish, common carp (*Cyprinus carpio*), and sunbleak (*Leucaspius delineatus*) in addition to crucian carp. The Vellavere pond measures 0.04 ha, with a water depth of 2 m, and contain crucian carp, goldfish, and tench (*Tinca tinca*). A total of 309 crucian carp were collected from the two ponds.

We transported the fish to the Centre for Limnology at Lake Võrtsjärv, where they were anaesthetized (benzocaine—50 mg l^{-1}), weighed, and photographed. A total of 228 fish, 114 from each pond, were randomised into six groups by size, transferred to their respective transport tank for recovery in water oxygenated by air-stones, and subsequently transported to the Utsali wetland area for release in the field. We sacrificed a total of 81 additional fish that were frozen for subsequent use in making skin extract. The skin obtained from these fishes (68 g) was divided into 11 batches for preparation of skin extract. In this case, the precipitate of each batch was re-homogenised in 150 ml water and centrifuged, and the supernatants were mixed before final dilution into the desired volume.

Field site and experimental protocol

We used six ponds in the Utsali wetland, a Nature protected area, for carrying out the field test. Each pond measured 5–6 m in diameter and was approximately 1 m deep. The experimental ponds had been produced by bombs, dropped by air planes into a previous Soviet military test site, and contained frogs, tadpoles and insect larvae, but no fish or known predators. A total of 38 fishes were released into each pond, representing 13 small fish (<1 g) and 6 larger fish (1.9–4.3 g) from Laeva pond, and 19 fish (4.5–14.0 g) from the Vellavere pond. Following release of crucian carp in the test ponds, the ponds were covered by bird nets to exclude potential predators.

The six experimental ponds were randomised in pairs to be exposed to either skin extract or control water (3 ponds × 2 exposure types). Each pair represented the two most similar sized ponds. We then added the frozen content of one ice-cube-bag (350 ml), either skin extract or control water, to each pond three times per week for 11 weeks from the middle of June to the end of August. The ice-cubes of each stimulus were spread evenly on the surface of their respective ponds. At termination of the experiment, fish were re-captured by large bottle traps, and brought back to the Centre for Limnology to be anaesthetized, weighed, and photographed.

During the course of the experiment, one of the three control ponds was observed empty of water during a draught period. Repeated trapping attempts at the end of the experiment revealed no surviving fish in that pond, and the pond was accordingly excluded from the analysis.

Analysis of fish morphology and statistics

We carried out measurements of fish gross morphology parameters on the photos by the use of ImageJ image analysis software (National Institutes of Health, USA). Body depth index was calculated from the formula: BDI = (body depth /fork length) x 100 (Holopainen and Pitkänen 1985). Fulton's condition factor (CF) was calculated from the formula: $CF = 100\,W \times L^{-3}$, where W = weight in grams and L = fork length in cm (Ricker 1975; Nash et al. 2006).

For testing differences between treatment groups, the Wilcoxon-Mann-Whitney test (Rank Sum Test) was used with two-tailed p-value for normal approximation (Siegel and Castellan 1988). Comparison of regression lines was carried out by the

linear regression procedure, using the computer program Statistix7 (Analytical Software, Tallahassee FL).

Results

The extracts used in the current study were prepared from a modified procedure involving centrifugation instead of filtering skin homogenates, and the presence of alarm signals hence had to be confirmed. When the stimuli was introduced for the first time in the laboratory experiments (Experiment 1 and 2), we therefore used thawed ice-cubes. In both experimental series, classical alarm behaviour was triggered with extracts made from skin of crucian carp. The results of the behaviour experiments will be reported elsewhere.

Experiment 1

The fish used in this experiment had a mean length of 60.6 mm (SD ± 3.42) and a mean weight of 3.2 g (SD ± 0.62) at the beginning of the experimental period. During the experiment these values increased to 65.3 mm (SD ± 4.47) and 5.3 g (SD ± 1.04), respectively.

Crucian carp, given a low feed ration and exposed to skin extract from conspecifics for a period of 50 days, increased the body depth index (BDI) from an average of 27.07 (SD ± 1.26) to an average of 29.10 (SD ± 1.10). In comparison, the low ration fed fish exposed to skin extract of brown trout increased the BDI from 27.74 (SD ± 0.90) to 29.12 (SD ± 1.15). The fish provided a high feed ration increased the BDI from 27.71 (SD ± 0.85) to 32.10 (SD ± 0.90) when exposed to skin extract of crucian carp, and from 27.17 (SD ± 0.98) to 32.85 (SD ± 1.05) when exposed to skin extracts of brown trout (Fig. 1a). No significant statistical differences were found in final BDI of fish exposed to different stimuli when given the same feed ration. However, for both types of stimuli, fish provided a high feed ration showed a significantly higher BDI at the end than those given a low feed ration ($p<0.001$), suggesting food intake as an important factor for the increase in body depth in crucian carp.

The body depth index of crucian carp seems to relate to the condition factor in a linear manner, as given by the data obtained at the beginning (Fig. 1b) and at the termination (Fig. 1c) of the experiment. The

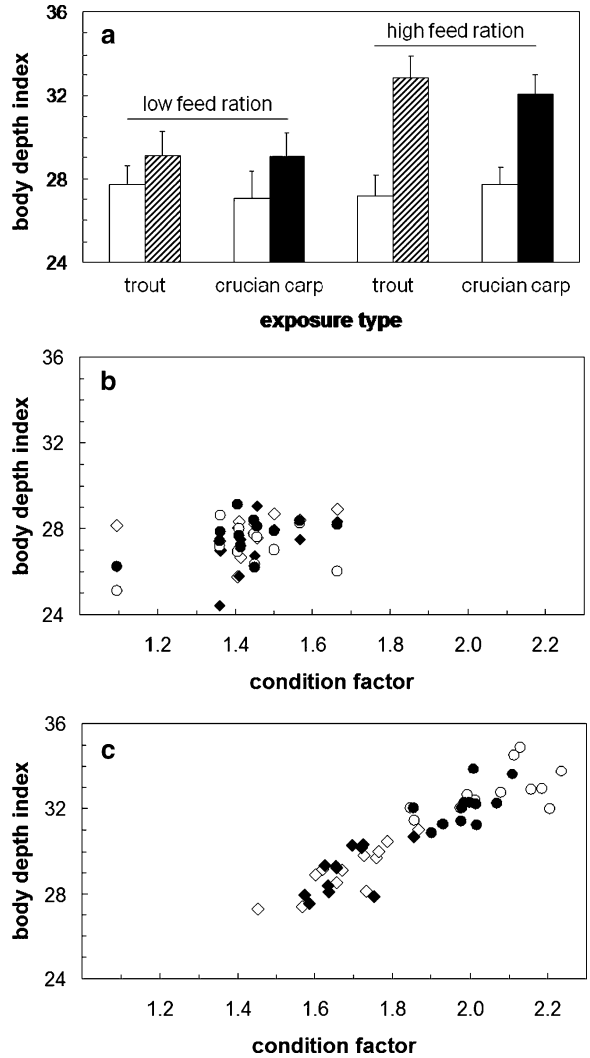

Fig. 1 **a** Body depth index (BDI) of crucian carp given either a low or a high feed ration, and exposed to the supernatants obtained from centrifuged skin homogenates of brown trout or crucian carp. Open columns: data from start; filled columns: data from end of experimental period. No statistical differences in final BDI levels were found between treatment groups within feeding regimes. **b** BDI as a function of condition factor (CF) of all fish at start and **c** at the end of the experiment. Diamonds: fish fed a low feed ration; circles: fish fed a high feed ration. Open symbols: fish exposed to extract of brown trout; filled symbols: fish exposed to extract of crucian carp

data from all fish combined follows a linear regression at the end, given by the equation:

$$y = 14.02 + 9.05x\,(R^2 = 0.84).$$

When comparing regression lines of fish exposed to skin extract of crucian carp versus skin extract of

brown trout, no differences were found neither in variances, slopes or elevations. However, for both BDI as well as CF, the individual changes from the start to the completion in high ration fed fish were found lower at a significant level when exposed to skin extract of crucian carp compared to skin extract of brown trout ($p<0.05$ and $p<0.01$, respectively), demonstrating that fish exposed to alarm signals grew less than the control fish (Table 1).

Experiment 2

The fish used in the experiment had a mean length of 63.8 mm (SD ± 5.22) and a mean weight of 3.8 g (SD ± 0.93) at start. During the experiment these values were increased to 66.1 mm (SD ± 5.92) and 5.3 g (SD ± 1.38), respectively.

Crucian carp exposed to skin extract of brown trout showed an increase in BDI during the experiment from a mean value of 27.34 (SD ± 1.03) to a mean value of 30.96 (SD ± 1.17), while the BDI showed an increase from 27.06 (SD ± 0.72) to 30.0 (SD ± 0.98) in fish exposed to skin extract of conspecifics (Fig. 2a). In comparison, crucian carp exposed to blank treatment (tap water) increased their BDI from 27.23 (SD ± 1.32) to 30.37 (SD ± 1.49), while fish exposed to skin extract of Arctic char increased their BDI from 27.09 (SD ± 1.08) to 30.26 (SD ± 1.31). No statistically significant differences were found for the increase in BDI of fishes exposed to any of the skin extracts compared to blank treatment (Table 2).

The relationship between BDI and CF of fish at the beginning of the experiment followed a linear regression (R^2=0.61), with no differences in variances, slopes or elevations between forthcoming treatment groups (Fig. 2b). At the end of the experiment, the total BDI to CF data revealed a less clear linear regression, as given by the equation:

$$y = 27.42 + 1.66x\,(R^2 = 0.13).$$

Statistical comparison of individual lines for treatment groups (n=12 for each group) revealed significant differences in slopes ($p<0.001$), but not in elevations or variances.

Experiment 3

Fish used in the experiment ranged between 25.2 and 94.7 mm in length and between 0.7 and 11.3 g in weight at the beginning of the experiment. At the end of the experimental period fish ranged from 36.0 to 96.8 mm in length and from 0.9 to 15.6 g in weight. Only one of the ponds treated with skin extract of conspecifics (pond A2) showed a significant increase in BDI of fish from the beginning to end of the experiment ($p<0.0001$; Fig. 3a). On the other hand, fish in the two control ponds that did not run dry during summer showed an increase in their BDI ($p<0.00001$; pond C2 and C3), suggesting a better growth in control ponds. Fish in the ponds C2 and A2 exhibited a significantly higher BDI than fish in the ponds A1 and A3 ($p<0.05$), while BDI of fish in pond C3 differed from those in C2 and A2 even further ($p<0.001$; Fig. 3a).

At the beginning of the experiment, crucian carp from the alarm and control ponds combined demonstrated a linear relationship of BDI to CF, following the regression line:

$$y = 17.63 + 7.45x\,(R^2 = 0.56;\ \text{Fig. 3B}).$$

Statistical comparison of regression lines for BDI/CF, calculated for each the two pond groups of fish

Table 1 Average changes (± S.D.) in individual body depth index (BDI) and condition factor (CF) of crucian carp during 50 days of exposure to the supernatant of centrifuged skin homogenates (Experiment 1). Skin was obtained from either brown trout (trout) or conspecifics (crucian), and fish were given either a low (0.03 g/fish/day) or a high (0.1 g/fish/day) feed ration. N=12 for each treatment group. Probability (p) for differences between treatments of similarly fed fish in statistical comparison was obtained by the Wilcoxon-Mann-Whitney test (two-tailed). n.s. = not significant (p>0.05)

Exposure type	Feed ration	BDI	p	CF	p
trout	low	1.38 (± 1.28)	n.s.	0.26 (± 0.17)	n.s.
crucian	low	2.03 (± 1.30)		0.25 (± 0.08)	
trout	high	5.68 (± 1.22)	<0.05	0.64 (± 0.11)	<0.01
crucian	high	4.38 (± 1.17)		0.50 (± 0.09)	

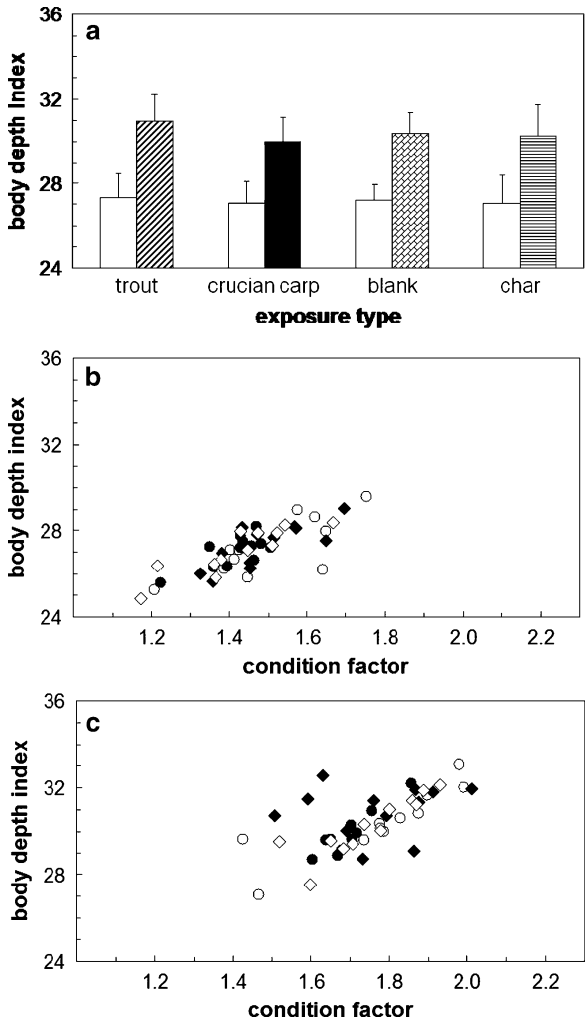

Fig. 2 **a** Body depth index (BDI) of crucian carp exposed to supernatants obtained from centrifugation of skin homogenates of brown trout, crucian carp or Arctic char, and tap water (blank). Open columns: data from start; filled columns: data from end of experimental period. No statistical differences in final levels of BDI were found between treatment groups. **b** BDI as a function of condition factor (CF) of all fish at start and **c** at the end of the experiment. Fish exposed to skin extract of: (♦) - brown trout, (●) - crucian carp, (◇) - Arctic char; and (○) - blank treatment

separately, showed no differences in variances, slopes or elevations. At the end of the experiment, the BDI/CF regression line of for all treatment pounds combined followed the linear regression:

$$y = 15.07 + 9.41x\,(R^2 = 0.73;\ \text{Fig. 3C}).$$

Again, comparison of separate regression lines revealed no differences in variances or slopes between treatments. However, elevations deviated at a statistically significant level ($p<0.05$). When tested separately, statistical significant differences were found between treatments for BDI as well as CF ($p<0.001$; both parameters; Wilcoxon), revealing that fish exposed to chemical alarm signals had increased less compared to control fish in both condition factor as well as body depth index.

Discussion

The data obtained in this study revealed that when crucian carp were exposed to chemical alarm signals from conspecifics for 50 days or more, the changes in body depth did not deviate from that found in fish exposed to control stimuli, neither in the laboratory nor in the field. The alarm signals were confirmed present in the supernatant obtained from centrifugation of skin homogenates, as given by behaviour tests carried out with thawed samples at start of the laboratory series. Further, for all stimuli applied, the BDI seems to relate to the CF in a linear manner.

Our results distinctly address two important processes of development of a deep body form in crucian carp. Firstly, the classical alarm pheromones triggering behavioural responses are not the ones possessing the primer pheromone effect (e.g., Karlson and Lüscher 1959; Wilson and Bossert 1963), meaning that they are not the causative agents for inducing morphological changes in crucian carp. Secondly, permanent presence of alarm signals do not result in increased body depth through indirect behavioural means, causing decreased activity, energy allocation and an higher overall growth, as suggested by some authors (e.g., Holopainen et al. 1997a; Vøllestad et al. 2004; Andersson et al. 2006; Johansson and Andersson 2009). Our results may, on the other hand, explain the lack of morphological responses from the "scratching with a knife" injury method by Brönmark and Pettersson (1994), because non-soluble tissue may not have been released with that method.

Crucian carp display extreme body forms in lakes with a diverse fish community, resulting mainly by the presence of piscivorous predators like pike and perch (Piironen and Holopainen 1988; Tonn et al. 1992; Poléo et al. 1995; Brönmark et al. 1995; Holopainen et al. 1997b). However, also in the absence of piscivorous, crucian carp develops higher

Table 2 Average changes (± S.D.) in individual body depth index (BDI) and condition factor (CF) of crucian carp during 50 days of exposure to the supernatant of centrifuged skin homogenates (Experiment 2). Skin was obtained either from brown trout (trout), conspecifics (crucian) or Arctic char (char), while frozen water was used as blank treatment. Fish were given a feed ration of 0.5 g/fish/day. *N*=12 for each treatment group. Probability (p) for differences between treatments and blank in statistical comparison was obtained by the Wilcoxon-Mann-Whitney test (two-tailed). n.s. = not significant (p>0.05)

Exposure type	BDI	p	CF	p
trout	3.62 (± 1.31)	n.s.	0.28 (± 0.20)	n.s.
crucian	2.94 (± 1.40)	n.s.	0.48 (± 0.54)	n.s.
char	3.17 (± 1.88)	n.s.	0.33 (± 0.21)	n.s.
blank	3.15 (± 1.85)		0.27 (± 0.21)	

CF's and deeper bodies in low-density environments, probably resulting from increased availability of food (Tonn et al. 1994). The data from our first experiment (Exp. 1) demonstrate that the feed ration influences both condition factor and body depth, and the relations between these parameters are linear. This supports the view of Brönmark and Miner (1992), where fish given a high feed ration developed a deeper body than fish on a low feed ration without predator cues present. Also in the field experiment (Exp. 3), when the fish were released into ponds at low densities with abundant natural food, BDI and CF showed a linear relationship. However, in neither case any signs of increased growth resulting from presence of alarm signals were noted, as proposed by Holopainen et al. (1997a). On the contrary, in both the environments we found that crucian carp exposed to chemical alarm signals grew less when compared to control fish. This implies that crucian carp stays more alert, and feed less when exposed to behavioural alarm signals, but no energy allocation takes place as a result of this stimuli alone. A suppressed growth due to alarm signal exposure seems to be a chemically induced parallel to the threat-sensitive foraging resulting from visual stimuli (e.g., Bishop and Brown 1992; Killen and Brown 2006).

The body depth index of crucian carp in mono-species ponds and lakes are generally found in the range of 26–34, while in multi-species communities with piscivorous predators they are commonly found between 40 and 50 (Holopainen and Pitkänen 1985; Holopainen et al. 1997a, b; Poléo et al. 1995).

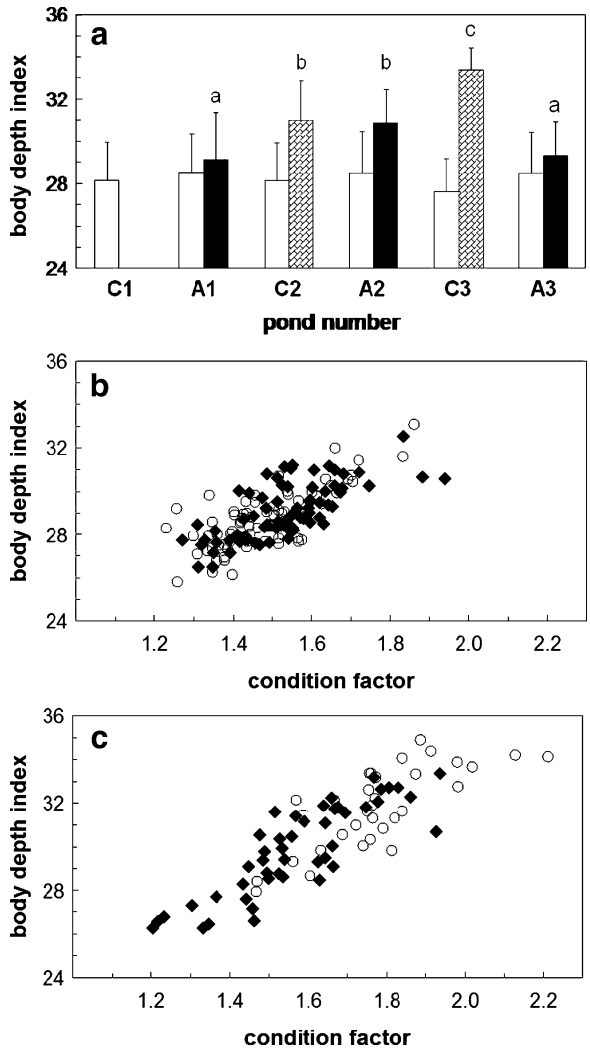

Fig. 3 **a** Body depth index (BDI) of crucian carp in ponds exposed to supernatants obtained from centrifugation of skin homogenates of crucian carp (A1-A3), or tap water (C1-C3); open columns at start and filled columns at the end of the experimental period. Different letters denote significant differences in final values. **b** BDI as a function of condition factor (CF) of all fish at start, and **c** at the end of the experiment. (♦) - fish exposed to skin extract of crucian carp; (○) - blank treatment

Crucian carp from high-density, mono-species ponds, transplanted to new and predator-free environments at low densities, grow faster during the first months than the original stock, most probably because of food abundance (Holopainen and Pitkänen 1985). However, such transplanted fish do not reach a BDI over 38, except for a few gravid females with distended bellies, even after four years at low population density with abundant food (Stabell and Durajczyk – unpublished

data). This suggests that crucian carp grow with regard to CF and BDI when food is abundant, and this takes place in a linear manner. However, in natural environments there appear to be an upper limit for such growth, and the extreme body depth found when predators are present must therefore result from other causes than linear growth alone. The logical implication is that the body-depth increase found after exposure to predator cues, as well as to filtered conspecific skin homogenates (Brönmark and Miner 1992; Brönmark and Pettersson 1994; Stabell and Lwin 1997; Chivers et al. 2008), must result from an additional set of primer pheromones (e.g., Wilson and Bossert 1963) present in the skin of these cyprinids.

We initially speculated that the lack of differences in BDI between crucian carp exposed to skin extracts of conspecifics versus brown trout, as found in Experiment 1, could be due to chemical substances in trout skin that resembled cyprinid alarm signals. In the work by Stabell and Lwin (1997), Arctic char skin extracts were used as control. We therefore brought in Arctic char of the same strain as previously used by Stabell and Lwin (1997) as an additional control species. The results of Experiment 2 made clear that the choice of reference species as donors for making control extracts was not the problem, because blank treatment gave similar results as the skin extracts. This conclusion is further strengthened by the fact that another non-cyprinid [i.e., swordtail (*Xiphophorus helleri*)], has successfully been applied as control species in morphological experiments with goldfish, using filtered skin homogenates (Chivers et al. 2008). The field experiment confirmed our previous results, and it seems clear that the lack of adequate morphological responses was due to the method of making skin extracts (i.e., because supernatants of centrifuged skin homogenates were used).

We suspect that the sources of chemical signals controlling morphology changes are found within the particles removed during centrifugation (i.e., in the precipitate). The tissue content of these particles may normally be metabolized by bacteria, either in the intestine of predators or in the water, resulting in water soluble cues. Such latent chemical signals, inducing morphology changes with the purpose of prey defences, have previously been found in other aquatic organisms, but have hitherto not been demonstrated in fishes (Hagen et al. 2002; Stabell et al. 2003; Jacobsen and Stabell 2004; Stabell 2005). Further studies are now underway to clarify the true origin of the chemical signals inducing morphology changes in crucian carp.

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