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PREFACE

This report stems from collaborative research undertaken by the South Australian Research and Development Institute (Dr.Ben Smith), the Pest Animal Control Cooperative Research Centre (Mr. Brad Tucker) and the University of Minnesota, U.S.A. (Drs. Sorensen and Sherman). We gratefully acknowledge a Post-Doctoral Travelling Fellowship from Land & Water Australia, which principally supported Dr. Smith's travel and living expenses in the USA. The Pest Animal Control CRC supported Mr. Tucker's travel and salary whilst in the USA. All laboratory equipment and supplies, together with Dr. Sherman's salary were kindly supported by the Minnesota Department of Natural Resources (MN DNR, US Grant #A74227 W.O.40/A48693).

While Dr. Sorensen was named Primary Investigator of this project, Dr. Smith and Mr. Tucker directed Experiment 1 and Dr. Sherman directed Experiment 2. Ms. Kathy Haskard (Senior Biometrician, BiometricsSA - SARDI) analysed the results from Experiment 1. This is an expanded version of a report that was submitted to MN DNR.

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EXECUTIVE SUMMARY

This project examined the possible use of flowing water and food-odour to attract common carp, *Cyprinus carpio*. Two experiments were conducted at the University of Minnesota, St. Paul campus (Aquaculture Laboratories), U.S.A.:

Experiment 1

Experiment 1 examined the behavioural response of small groups of carp (two or five fish) to four current flow-velocities (Basal-control, Low, Medium and High) at two water temperatures (16 and 21°C). Initial mapping confirmed the near identical character of the pattern and velocity of flow in each of two circular experimental tanks, and facilitated the division of each tank into four flow-zones: 'No-Flow', 'Transition', 'Flow' and 'Upstream'. The behavioural response of carp to each combination of flow, density and temperature was determined from video recordings filmed remotely via overhead infrared camera. Recorded behavioural responses included data on position (relative time spent in each flow-zone), rheotaxis (relative time spent orienting toward the current), and movement to the 'Upstream' zone (relative time spent attempting to reach the source of the current flow).

We found that carp consistently moved from still water into flowing waters, and that this movement increased proportionally with an increase in current-flow. Once the carp entered flowing waters, they exhibited rheotaxis at all velocities tested (6-32 $cm.s^{-1}$), and the proportion of carp facing upstream increased with increasing flow. Whilst visual evidence of attraction to the source of the current in-flow was noted, particularly in warm water, variability between treatments precluded statistical confirmation.

Experiment 2

Experiment 2 examined the behavioural responses of groups of three male carp, to food odour (Control and Odour) released at two current-flows (Basal-control and Flow) within a laminar-flow fluvarium (3.6 m x 1.1 m). The experimental area of the fluvarium was divided into upstream and downstream areas, relative to flow, and the upstream area was further divided into 3 sub-areas (Left, Centre, Right) to evaluate whether carp spent more time in the central sub-area where the flow/odour was released. Recorded video footage for each flow x odour combination was scored for three behavioural responses of carp: position in tank, success at finding the odour source and initial time taken to find the odour source.

In support of the results from Experiment 1, carp exhibited a rheotactic response at the lowest current-velocity tested (3 $cm.s^{-1}$). Attraction was especially strong when current-flow and odour were combined; carp were swift in their approach and they repeatedly probed at the source of the odour. In the absence of flow, odour alone was found to stimulate the movement of carp from a grassy shelter area, which was their preferred habitat in the absence of any stimuli.

In conclusion, the combined use of flowing water and odour shows considerable promise for attracting common carp. Not only did specific combinations of flow and odour effectively ‘pull’ carp from their preferred habitats, they also persuaded carp to spend longer in the flow zone and repeatedly swim toward the source of the flow/odour. Thus, flow and odour could facilitate the trapping and removal of common carp from natural systems. Importantly, this approach should prove feasible in locations such as Australia, where the availability of water for environmental rehabilitation is limited, because significant attraction was still noted at low current-flow velocities. Future studies should examine higher flows and different odours, including pheromone attractants

INTRODUCTION

Common carp (*Cyprinus carpio* L.) are a declared pest fish, and a formidable invader of degraded water-bodies in south-eastern Australia (Koehn, 2004). Where they are highly abundant, carp are implicated in the destruction of benthic and riparian habitats and declines in native biodiversity. The effects of carp are most obvious in shallow wetlands where their bottom feeding behaviour disturbs large quantities of sediments, nutrients, and rooted aquatic plants, thereby causing eutrophic conditions unsuitable for other fishes, birds, or plants (Robertson *et al.*, 1997; Lougheed *et al.*, 1998; Angeler *et al.*, 2002; Smith, 2005). Reclamation of degraded watersheds has been hindered by a lack of practical means to target and remove carp. Ongoing research at the University of Minnesota suggests that chemical attractants (pheromones and food odours) have potential for use in common carp control (Sorensen & Stacey, 2004). However, to be effective these attractants must be potent and applied in the most effective manner possible. This report addresses the possibility that current flow might be used to attract carp, especially in conjunction with chemical attractants.

It is well established that many freshwater fishes rely heavily on chemical cues to find food, mates, conspecifics, and specific locales (Sorensen & Caprio, 1998). Carp appear to be no exception as gauged from numerous studies of its close relative the crucian carp (*Carassius carassius)* and goldfish (*Carassius auratus*) (Sorensen, 1992; Sorensen & Stacey, 1999). However, a variety of evidence suggests that fish find locating the source of an odour in still-water very difficult (Kleerekoper, 1967). Indeed, many fishes simply do not appear capable of tracking diffuse odour plumes in still waters and those that can, rely on kineses (undirected changes in movement pattern stimulated by changes in concentration) which are typically inefficient (Fraenkel & Gunn, 1961; Dusenbury, 1992). This situation contrasts with that created by the presence of current flow, which has two effects:

1) It provides a directional cue that at least some fishes can follow using their lateral line system (Montgomery *et al.*, 1997);
2) It generally creates a better-defined odour plume, which also is easier for fish to follow (Dusenbury, 1992).

Rheotaxis is defined as behavioural orientation to (and within) water currents (Montgomery *et al.*, 1997). When exhibiting a rheotactic response fish align their bodies with the current flow, behaviour that results in 'attraction' (Fraenkel & Gunn, 1961; Kleerekoper, 1967; Zimmer-Faust *et al.*, 1995; Montgomery *et al.*, 1997). Attractive odours are believed to release a rheotactic response in many species of migratory fishes (Kleerekoper, 1967; Sorensen, 1986) but as yet this has not been shown in the carp.

Some species of fish are attracted to certain flow regimes, even in the seeming absence of odour (Chan *et al.*, 1997; Coutant, 2001). Although this phenomenon can be complicated by fish size and physiological condition, responses are often strong. Indeed, most dam structures in North America are constructed with fish ladders that employ 'attractant flows' (Coutant, 2001). Notably, flows are relatively easy and inexpensive to manipulate. Unfortunately, we have little understanding of the topic for most fish including the common carp. However, recent evidence suggests that carp may be positively rheotactic under certain (largely undocumented) flow conditions and 'jumping traps' have been placed at various dam structures (Stuart *et al.*, 2003).

The possibility that flow and chemical stimuli might synergize each other's attractive properties opens up the possibility of using them together to attract carp into traps. Juvenile carp are sensitive to chemical food stimuli at 60 mm total length and will show full development of food searching behaviour at 1 year of age (Kasumyan & Ponomarev, 1990). Notably, an interaction between flow and food odour has been documented in the goldfish (Rand & Kleerekoper, 1975). This study aimed to conduct pilot studies to determine if water flow might be useful to attract mature carp either in isolation, or in combination with an odour stimulus. We asked four questions:

1) When offered a choice of still or flowing water, will carp choose the latter? If so, is the extent of the preference related to flow velocity?
2) Do carp exhibit positive rheotaxis in the absence of odour?
3) Are carp attracted to the source of current inflow?
4) Are carp more efficient at finding an odour source in flowing water than in still water?

Questions 1-3 were addressed by Experiment #1, while question 4 was addressed by Experiment #2. Below, we describe each experiment and then present a brief, general discussion.

EXPERIMENT 1. Exploring the response of carp to current flow

METHOD

Design

Experiment #1 addressed Questions 1-3 by examining the responses of carp to four treatment flow velocities (Basal-control, Low, Medium, High) at two water temperatures (cold, 16.2 ± 0.3°C; warm, 20.6 ± 0.3°C) using two densities of fish (two or five).

Synchronous trials (a trial incorporates all four treatment flows) were conducted in two tanks over 12 days, with one trial per tank per day. Each trial used independent fish (replaced daily), and blocks of four trials (2 tanks over 2 days) were conducted for each temperature x density combination. The daily order of flows per tank was set according to a replicated 4 x 4 Latin-square design (Table 1A), and the specific flow conditions tested are shown in Table 1B. Each set of four trials (incorporating 16 ‘runs’) is denoted as a ‘square’. The treatments were applied in this experiment as in a split-plot experiment - the squares were wholeplots, with the wholeplot treatment Temperature x Density (TempDens) being applied to all the runs in a whole square. The 16 individual runs within a square (4 trials x 4 times) are the subplots, and the subplot treatment, denoted simply as Treatment, is the flow rate: Control (C), Low (L), Medium (M) or High (H).

Plumbing constraints meant that all of the warm water trials were conducted first (days 1-8), followed by all of the cold water trials. The latter cold-water trials were conducted with only 2 fish per tank, because of observations from the warm-water trials that the largest fish were influencing the behaviour of the remainder of the group; which tended to follow the leader.

Animals

Young carp (≈100 mm total length, TL) were purchased from Osage Catfisheries (Missouri, U.S.A. - carp are from a Mississippi River stock) and reared for one year under laboratory conditions that promoted fast growth and precocious maturation (photoperiod, 18 h light: 6 h dark; water temperature, 21°C; feeding, Tetramin® flakes twice per day *ad libitum*). All carp were sexually mature but no attempt to distinguish the sex of individuals prior to experimentation was made. Total lengths ranged from 15-23 cm (mean 18±2 cm S.D.).

Apparatus

Trials were conducted in two identical flat-bottom circular tanks (1500 L, 1.5 m diam.) at a water depth of 20 cm and filmed remotely via a high-resolution overhead camera (Bosch Dinion LTC0355), linked to a Super VHS recorder. The tanks were enclosed by black plastic to shield the fish from external disturbance and to maintain the desired 16 h light: 8 h dark photoperiod; simulated day-light was established via two indirect 25 W dim-lights set to emit c. 65 lux each (measured at 10 cm from the light globes). One infrared light (Vitek VT-IR1/110) was also positioned near each camera and a background of white plastic was taped to the bottom of each tank to compensate for the darkened conditions.

Table 1A. Summary table indicating the design for Experiment 1, which incorporated a split-plot and Latin square design.

Warm water (21°C) - 5 & 2 fish				Daily order of Treatment Flows			
Day	Trial Number	Tank Number	Density of Fish	1	2	3	4
1	1	1	5	C	L	M	H
1	2	2	5	L	M	H	C
2	3	1	5	M	H	C	L
2	4	2	5	H	C	L	M
3	1	1	2	C	L	M	H
3	2	2	2	L	M	H	C
4	3	1	2	M	H	C	L
4	4	2	2	H	C	L	M
5	5	1	5	C	L	M	H
5	6	2	5	L	M	H	C
6	7	1	5	M	H	C	L
6	8	2	5	H	C	L	M
7	5	1	2	C	L	M	H
7	6	2	2	L	M	H	C
8	7	1	2	M	H	C	L
8	8	2	2	H	C	L	M

Cold water (16°C) - 2 fish only				Daily order of Treatment Flows			
Day	Trial Number	Tank Number	Density of Fish	1	2	3	4
9	1	1	2	C	L	M	H
9	2	2	2	L	M	H	C
10	3	1	2	M	H	C	L
10	4	2	2	H	C	L	M
11	5	1	2	C	L	M	H
11	6	2	2	L	M	H	C
12	7	1	2	M	H	C	L
12	8	2	2	H	C	L	M

Table 1B. Summary table indicating the treatment flows and their companion flow rates and current velocities within the 'No-Flow' and 'Flow' zones.

	Flow rate at	Current velocity in tank (cm.s^{-1})	
Treatment flow	tank inlet (L.m^{-1})	'No Flow' zone	'Flow' zone
Control	2	0	0
Low	25	0	6-10.5
Medium	45	0	10.5-18.5
High	70	0	19-32

Two 40 cm diameter buckets with drainage holes cut at a height of 20 cm were taped over the central drains to maintain the desired water depth. Water was introduced at the perimeter of each tank via a downpipe with a 15 x 0.5 cm vertical slit at the bottom, pressured by an external pump. Two in-line flow meters facilitated the application of precise and replicable flow rates. Experimental fish were constrained to one half of the tanks by a flow-through mesh barrier, and solid clear-Plexiglass barriers, extending halfway into the tanks from the central buckets, provided shelter from flow (and thus a choice). Aeration was provided near the water inlets (Fig. 1A).

Flow mapping

To confirm that the experimental tanks were indistinguishable in terms of their flow patterns and velocities, and thus, that it was possible to use two tanks to expedite the experiment, current velocities were mapped along two ‘spokes’ per tank (spokes A & B, Fig. 1A), at each of four flow rates (2, 25, 45 and 70 $L.m^{-1}$; hereafter Basal, Low, Medium and High). Spokes extended from the central bucket to the tank edge, at angles of 45° and 135° from the mesh barrier. For each flow rate and spoke, three replicate measurements were taken at seventeen intervals using a portable flow meter (Model 2000 Marsh-McBirney Flo-Mate™), at a depth of 10 cm (Fig. 1A). Current velocities were compared between tanks and spokes via a 4-way ANOVA. ‘Tank’, ‘Spoke’, ‘Distance’ (from edge of tank) and ‘Flow Rate’ were factors in the model, which also included 5 key interaction terms (out of 11 possible interactions). No interactions were significant, and as anticipated, only flow rate and distance from the tank edge affected flow velocities (Table 1C); due to the circular shape of the tank, current velocities decline exponentially with distance from the tank edge (Fig. 1B). Thus, the flow mapping data was pooled among tanks and spokes for each flow rate, and average values were plotted (Fig. 1B).

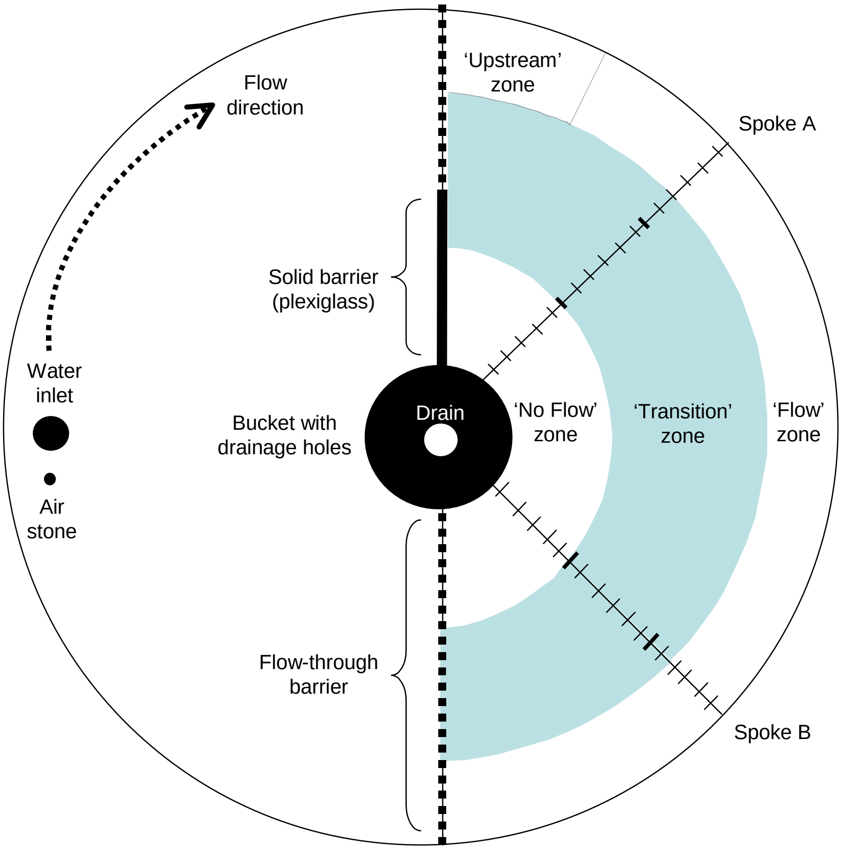

Figure 1A. Experimental set-up showing position of the water inlet, bucket with drainage holes drilled at a height of 20cm, central drain, flow-through barrier, solid barrier (plexiglass), two flow 'zones', and spokes A and B along which measurements of water velocity were taken to compare flows within and between tanks. Seventeen spot measurements were taken along each spoke, at ≈ 3.15 cm intervals from the edge of the tank.

Table 1C. Analysis of Variance testing that current velocities at each flow rate (Low, Medium, High) are not significantly different between Spokes or Tanks.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F-ratio
Model	71	10267.46	144.612	13.175
Error	132	1448.91	10.977	Prob > F
C. Total	203	11716.37		<0.0001

Effect Tests

Source	DF	Sum of Squares	F-ratio	Prob > F
Tank	1	0.23	0.021	0.886
Spoke	1	1.81	0.165	0.686
Distance	16	9098.51	51.807	<0.0001
Flow Rate	2	1123.42	51.174	<0.0001
Tank*Flow Rate	2	1.74	0.080	0.924
Tank*Spoke	1	1.52	0.138	0.711
Tank*Distance	16	13.07	0.074	1.000
Spoke*Distance	16	23.57	0.134	1.000
Tank*Spoke*Distance	16	3.59	0.021	1.000

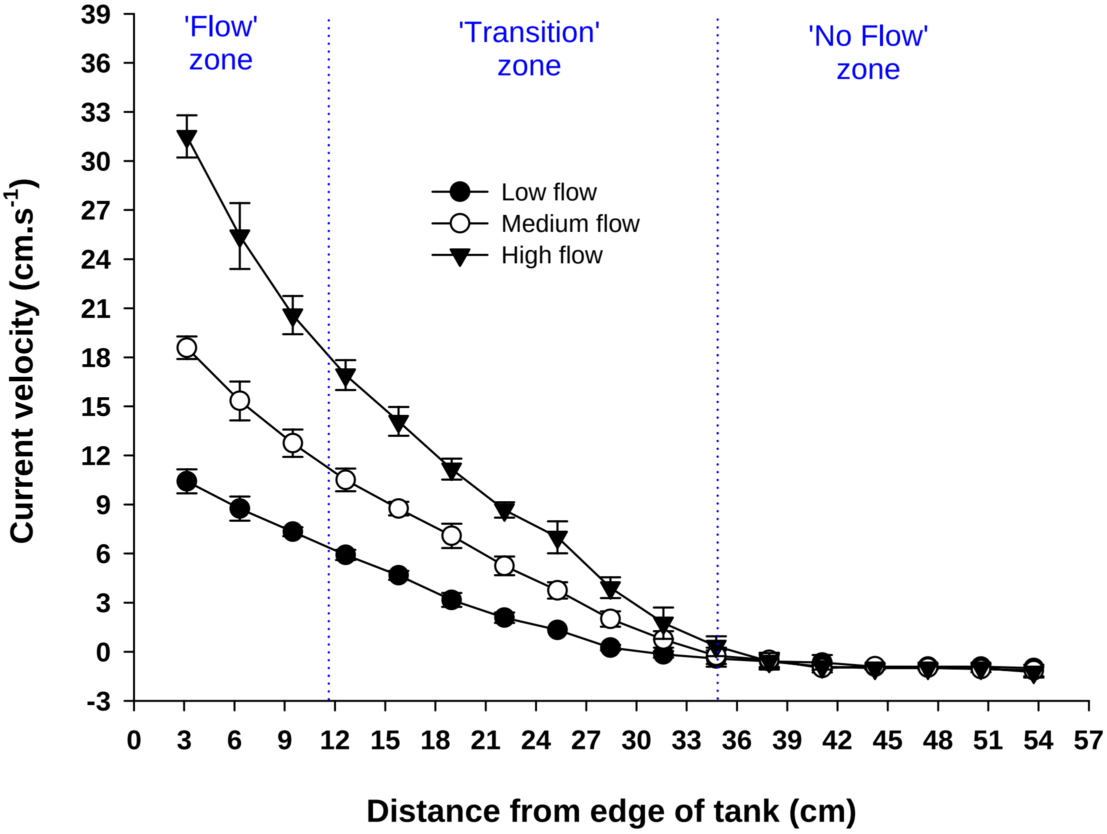

Figure 1B. Averaged data (pooled for Tanks 1 & 2, and Spokes A & B) showing the range in current velocities generated within each flow zone (‘No-Flow’, ‘Transition’ and ‘Flow’) for each of three treatment flow-rates (Low, Medium and High). Current velocities decrease exponentially with distance from the edge of the tank. Current velocities at the Control flow rate of 2 L.m^{-1} are undetectable in each zone. A key to symbols and S.E. bars are included.

Protocol

The afternoon prior to an experimental trial, carp were fed, measured (TL, mm) and transferred into each test tank from their holding tanks (250 L, 75 cm diameter, well-oxygenated recirculating water). An attempt was made to match the size of individuals allocated to tanks, as it has been observed that large ‘leader’ carp can influence the behaviour of cohabiting individuals in laboratory environments (Dooland *et al.*, 2000; Champion *et al.*, 2001). Fish were acclimated overnight at the desired photoperiod (16 h light: 8 h dark), light intensity (≈ 1 lux at the water surface) and Basal (control) flow rate of 2 L.min^{-1} (<0.5 cm.s^{-1}). The base-flow maintained stable water levels and temperatures.

The next morning, current-flow velocities were set alternately in each tank, until all four-treatment velocities had been tested. After a desired flow velocity was established, the fish were allowed to acclimate for 20 minutes. The next 10 minutes were observed remotely and recorded on a Super VHS recorder. At the completion of each flow-velocity, flows were returned to Basal (control), and fish were allowed to rest for 30 minutes. At the end of each day, the two tanks were drained, cleaned and refilled before new fish were introduced and acclimated overnight.

Recorded data

We analysed all 10-minute video recordings using a Panasonic AG1980 Super VHS desktop editor, linked to a P.C. and motion analysis software (Peak Motus version 6.1, Peak Performance Technologies Inc.). For each recording, we scored the position and orientation of test fish every 30 seconds. This enabled the collation of data on three behavioural responses to each combination of current-flow velocity, water temperature and fish density:

1) Position in flow: Relative time spent in each flow-zone (Fig. 1A).
2) Rheotactic response: Relative time spent orienting toward the current in the ‘Transition’ and ‘Flow’ zones.
3) Attraction to water at the ‘in-flow’ zone: Relative time spent attempting to reach the source of the current flow. In this instance, fish were required to be within the ‘Upstream’ zone *and* exhibiting positive rheotaxis (orienting into the current).

For all recorded behaviours, a fish was deemed to be within a zone if the tip of the snout was within that zone; fish were orienting toward the current if their body axis was aligned to within approximately 290° and 70° to the current.

Statistical Analysis

Position data

For the position data, the proportions of carp in each flow-zone were analysed in two ways:

1. Weighted Analysis of Variance (weighted ANOVA): proportions of fish per zone were first arcsine square-root transformed to conform to the parametric assumption of equal variances. Data was then weighted to account for differences in variance caused by the two densities of fish used.

The effects of the Temperature x Density (TempDens) combinations were tested on each experimental unit (the 'squares'). When an effect was found to be statistically significant ($\alpha = 0.05$ for all tests), it was investigated using appropriate contrasts or comparisons amongst its levels. For the TempDens effect (2 df) the obvious comparisons were between warm water with 5 fish (w5) and warm water with 2 fish (w2), and between w2 and cold water with 2 fish (c2). For the treatment effect, a contrast comparing the Control with the average of Low, Medium and High flows was made - Control is qualitatively different from the three flowing treatments, because there is no flow in any zone. If this was statistically different, a comparison was made between Low, Medium and High flows, partitioning the effect (remaining 2 df) into a linear contrast and the deviation from linear.

2. Categorical data analysis, assuming a multinomial distribution for the three counts: If the treatment effect (with 3 df) was found significant, it was partitioned in the same way as for the weighted ANOVA: 1 df to compare Control with Flowing (L, M & H combined), 1 df for a linear trend among L, M and H, and 1 df for the deviation from linear. When an effect was found to be significant, the parameter estimates were used to indicate which levels of the factors were different.

Rheotaxis and Movement data

Logistic regression with quasi-likelihood was used to compare the number of fish that were orienting into the current in each of the Transition, Flow and Upstream zones, as proportions of fish within those zones (answering Q2 & Q3 of the Introduction). The same provisos apply here as for the categorical analysis for the position data.

RESULTS

Position in flow and flow preference

Weighted analysis of variance on the proportions in the flow zone suggested no significant interaction between flow velocity and TempDens (P = 0.194), a significant flow-velocity effect (P = 0.015), and no significant main effect of TempDens (P = 0.465). Partitioning the flow-velocity effect into Control versus Flowing, then linear and quadratic components among the flowing treatments, suggested a significant difference (P = 0.007), with a weakly significant linear trend among those with some flow (P = 0.071; deviations from linear not significant, P = 0.651).

Similar results were obtained for the proportions of carp in the No-Flow zone; there was no interaction between flow velocity and TempDens (P = 0.563), a significant flow-velocity effect (P = 0.026), and no significant main effect of TempDens (P = 0.601). The Control was only weakly significantly different to flowing treatments (P = 0.068), but there was a clear linear trend among those with some flow (P = 0.016; deviations from linear not significant, P = 0.486). However, analysis of the proportions in the Transition zone suggested a significant interaction between flow-velocity and TempDens (P = 0.009). Logistic regression analysis of the same three position variables, using quasi-likelihood to adjust for over-dispersion, gave consistent results.

The above results are well-illustrated in Figures 1C-D. At the onset of experimentation and during Basal-control flows, fish moved throughout the tanks without pattern or preference for any region. Yet, with the onset of flow, the carp spent a greater proportion of time in the 'Flow' zone, and among the flowing treatments, increasing flow tended to increase the proportion. Associated with this was a concurrent reduction in the proportion of time spent in the 'No-Flow' zone (Fig. 1C). Whilst observation suggested that paired fish showed a stronger response to flowing water than did groups of five, and that a stronger response was observed in warm water, the analyses show no evidence of this.

The interaction between flow-velocity and TempDens in the Transition zone was due to disparities among the responses of w5 and c2, and w2 TempDens combinations. That is, the response of w5 and c2 fish were similar to each other and similar for all flow rates, but w2 displayed a very different pattern as the flow velocity was changed from Control (w2 higher) to flowing (w2 lower, with little difference between flow rates) (Fig. 1D).

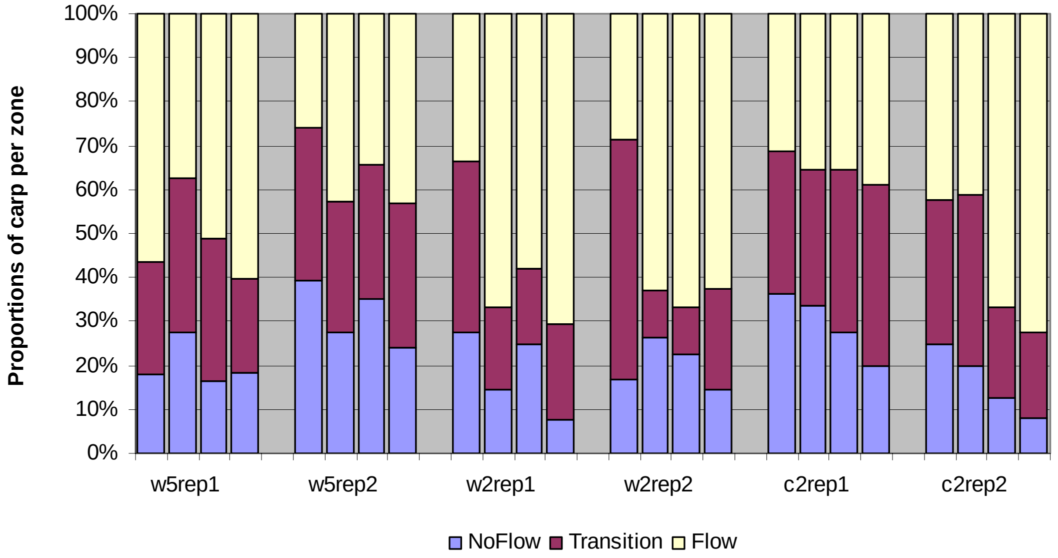

Figure 1C. Relative proportions (%) of fish in each flow-zone (Flow, Transition, No-Flow), for each split-plot replicate of the three Temperature x Density combinations; w5 = warm water with five fish; w2 = warm water with two fish; c2 = cold water with two fish. Each group of four columns represents the Control, Low, Medium and High flow treatments respectively.

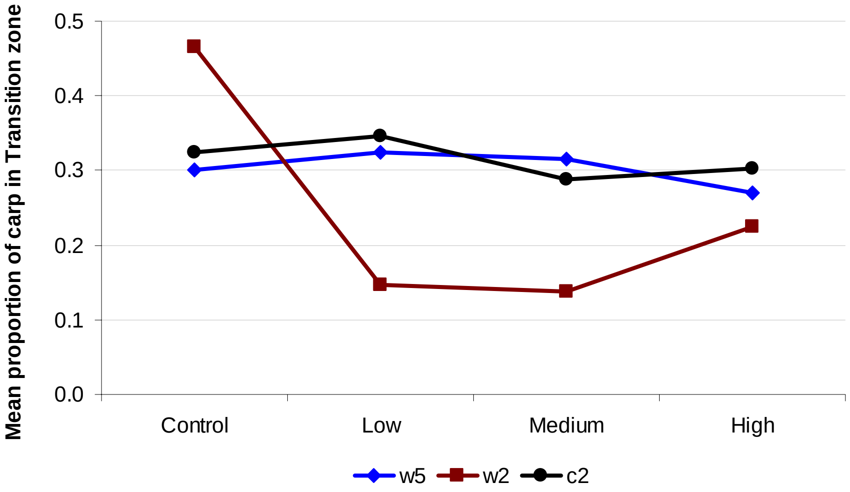

Figure 1D. Mean proportion of carp in the Transition zone by Flow-velocity, for each TempDens combination. w5 = warm water with five fish; w2 = warm water with two fish; c2 = cold water with 2 fish.

Rheotactic response & attraction to current in-flow

Logistic regression (using quasi-likelihood to allow for over dispersion) showed that, for the number of fish facing upstream in the Transition zone, out of the total number of fish in that zone, there was no significant TempDens x Flow velocity interaction (F = 0.47, df = 6, 42, P = 0.829) but a significant main effect of Flow velocity (F = 20.4, df = 3, 48, P = <0.001). The treatment effect can be attributed to a difference between Control and Flowing (F = 52.56, df = 1, 48, P = <0.001, with higher proportions facing upstream when there is flow) plus a linear trend among the Flowing treatments (F = 8.59, df = 1, 48, P = 0.005, with positive slope; non-linear component, F = 0.05, df = 1, 48, P = 0.831).

A similar analysis for the number of carp facing upstream in the Flow zone, out of the total number in that zone, shows that there is no statistically significant TempDens x Flow velocity interaction effect (F = 0.64, df = 6, 45, P = 0.699) but a significant main effect of Flow velocity (F = 40.7, df = 3, 51, P = <0.001). The treatment effect can be attributed to a difference between Control and Flowing (F = 110.1, df = 1, 51, P = <0.001), plus a linear trend among the Flowing treatments (F = 11.75, df = 1, 51, P = 0.001, with positive slope; non-linear component, F = 0.24, df = 1, 51, P = 0.624).

For the proportion of carp facing upstream at the fence (within the ‘Upstream’ zone), out of the total number facing upstream in the Flow zone, there was no significant TempDens x Flow velocity interaction effect (F = 3.37, df = 6, 44, P = 0.842) nor a significant effect of Flow velocity (F = 10.1, df = 3, 44, P = 0.27). Testing Control versus flowing showed no significant difference (F = 0.2.34, df = 1, 52, P = 0.132). This may be due in part to increased variability caused by the small number of carp involved – we were down to a subset (at the fence), of a subset (facing upstream) of a subset (in the Flow Zone). Further, there is a significant difference between trials within squares for this variable (F = 4.28, df = 18, 71, P = <0.001). This suggests that there is considerable variability between trials, which could mask or distort any apparent TempDens effect.

Examination of Figures 1E-F support the analyses of rheotactic behaviour outlined above. They illustrate that the proportions of carp facing upstream in the Transition and Flow zones are higher when there is flow than for Control, and that the linear trend as flow increases is upwards i.e., the stronger the flow, the greater the proportion of carp that are facing upstream. Indeed, the proportions facing upstream for the Transition and Flow zones increased from 14% and 49% for Control, to 43% and 82%, 49% and 94%, and 58% and 94% for Low, Medium and High flow respectively.

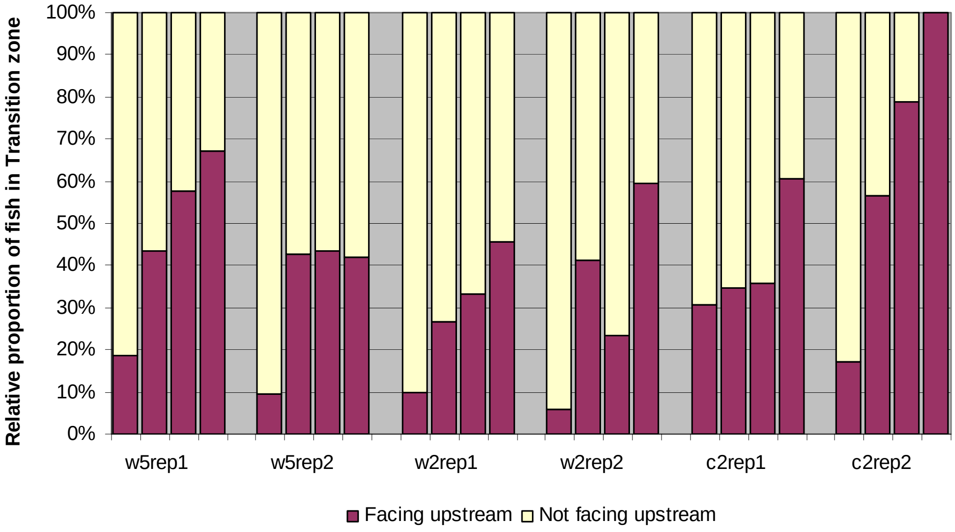

Figure 1E. Orientation of fish in the Transition zone for each split-plot replicate of the three Temperature x Density combinations; w5 = warm water with five fish; w2 = warm water with two fish; c2 = cold water with two fish.

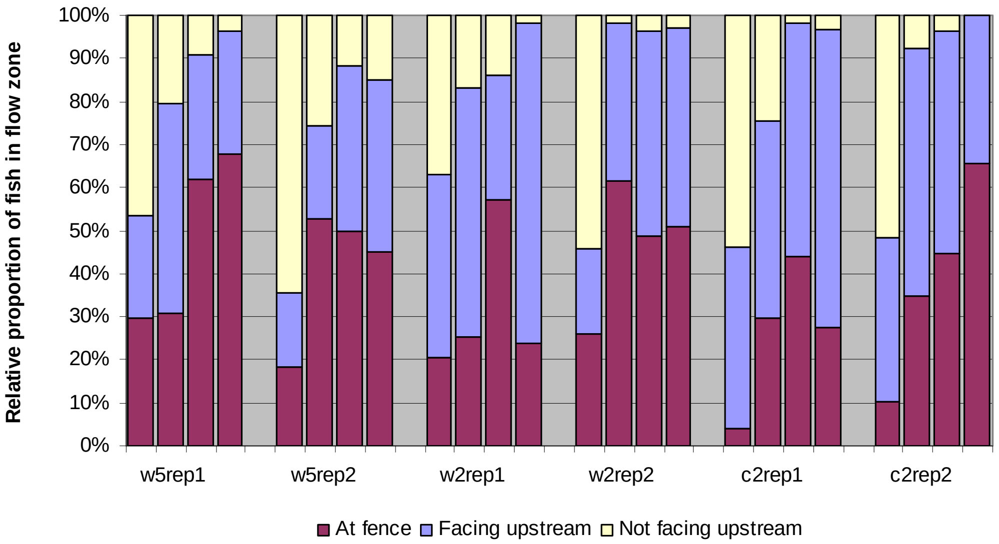

Figure 1F. Orientation and movement of fish in the Flow zone for each split-plot replicate of the three Temperature x Density combinations; w5 = warm water with five fish; w2 = warm water with two fish; c2 = cold water with two fish. The proportion 'At fence' represents those fish that were facing upstream and positioned within the 'Upstream' zone.

DISCUSSION

When offered a choice of still or flowing water carp preferred flowing water, and the greater the flow velocity the more likely they were to be in the current. This response was accompanied by persistent orientation into the flow (even at the slowest flow conditions that we could reliably create in our tanks, 6 cm.s^{-1}), and thus may be considered to be rheotaxis. Rheotactic- and attraction responses appeared to be modulated by social conditions (paired fish responded most strongly) and enhanced by temperature, but this was not supported statistically. Fish did not appear to fatigue, even at the highest velocities tested (32 cm.s^{-1}; approx. 2 body length.s^{-1}), although fish did not spend notable periods of time at the water inflow and could rest periodically in the No-Flow zone.

EXPERIMENT 2: Exploring the response of carp to flow *and* odour

METHOD

Design

Experiment #2 addressed Question 4 by examining the responses of groups of three male carp[1] to two flow velocities (‘No Flow’, <0.1 cm.sec^{-1}; ‘Flow’, 3 cm.sec^{-1}) and two concentrations of odour (‘No Odour’, well-water only; ‘Odour’, filtered solution of Tetramin® flakes in water). Each of the four flow x odour combinations or ‘runs’ (No Flow x No Odour; No Flow x Odour; Flow x No Odour and Flow x Odour), were trialled once per day over 12 days (typically, 10:00 to 17:00 each day) in a single laminar-flow fluvarium. The daily order of testing was such that the two flow conditions were alternated in a balanced manner and the odour trials were conducted last (Table 2A). The specific flow conditions tested are shown by Table 2B. Each group of three experimental carp was replaced daily.

Animals

Mature males (identified via milt extraction) from Experiment 1 were used in this experiment after a 3-week respite. Total lengths ranged from 14-23 cm (mean 18±0.41 cm S.E.) and weights ranged from 40-117 g (mean 68±3.2 g).

Apparatus

Experiments were conducted in a single laminar-flow fluvarium (3.6 m x 1.1 m), at a water depth of 10 cm (Fig. 2A). A shelter area (hereafter, termed the ‘gated area’) was located at the downstream end, with a central 18 cm opening and artificial weeds, in which carp preferred to reside in the absence of an odour/flow stimulus. Warm water (20.5 ^{o}C) was introduced into the upstream section of the fluvarium from two underlying inter-connected 10,000 L reservoir tanks via a series of valves and pumps (10-500 L min^{-1}). This water passed through a series of baffles and

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[1] Male carp are relevant here because they will be they focus of trapping and removal efforts at carp recruitment hot spots. Reducing the number of wild male carp at select sites within the Murray-Darling Basin will improve the prospects for Daughterless males in propagating the Daughterless gene.

collimators to create a laminar flow regime within the experimental area (140 x 109 cm), which was bounded by a series of mesh flow-through barriers. A peristaltic pump (50 $ml.min^{-1}$) and pipette positioned in the centre of the upstream grid, enabled 1) the introduction of test odours into the fluvarium, and 2) testing of the mixing dynamics of the fluvarium using Rhodamine dye (Table 2B). This enabled the division of the tank into four main areas for data analysis: Gate (within gated area), Left, Right and Centre (where the odour was added).

Table 2A. Experimental design of Experiment 2. FC = Flow + Control; NFC = No-Flow + Control; NFO = No-Flow + Odour; FO = Flow + Odour.

Day (Trial Number)	Treatment Order			
	1	2	3	4
1	FC	NFC	NFO	FO
2	NFC	FC	FO	NFO
3	FC	NFC	FO	NFO
4	NFC	FC	FO	NFO
5	FC	NFC	FO	NFO
6	NFC	FC	FO	NFO
7	FC	NFC	NFO	FO
8	NFC	FC	NFO	FO
9	FC	NFC	NFO	FO
10	NFC	FC	NFO	FO
11	FC	NFC	FO	NFO
12	NFC	FC	NFO	FO

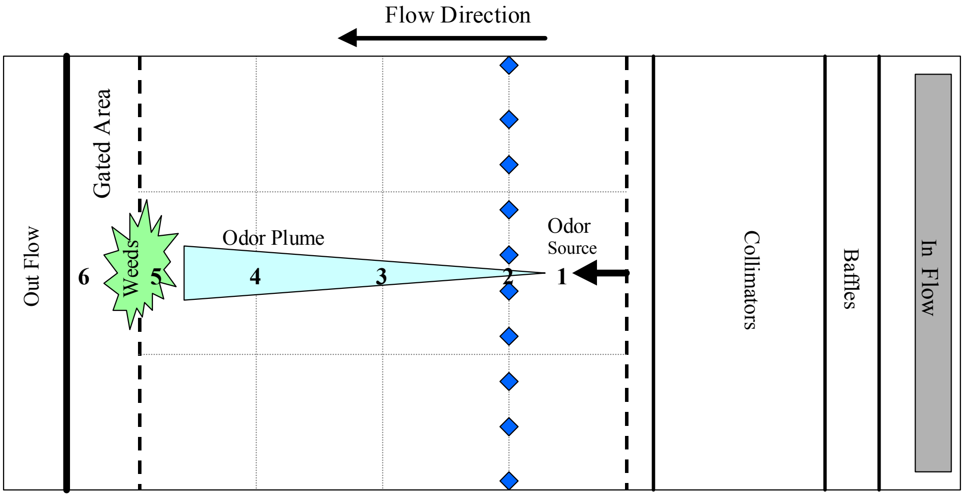

Figure 2A. Experimental set-up. The dark black dashed lines are flow-through barriers used to restrict fish movement. During initial testing of flow rates and plume dynamics under different flows, average velocities and concentrations of Rhodamine dye were measured at the locations denoted by the numbers 1-6. Flow measurements were also taken at 10 points (blue diamonds) along each vertical, lightly-dashed line.

Table 2B. Summary table describing the odour plume (shape and time to gated area) under each flow rate, and the average flow velocities within the experimental area.

Treatment (flow)	Odour - time to gated area (ave ± S.E. sec)	Shape of odour plume	Current-velocity (ave ± S.E. $cm.s^{-1}$)
None	208 ± 16.2	Wide Cone*	0 ± 0
Flow	20 ± 1.54	Narrow Cone**	3 ± 0.001

* The wide cone was ≈ 3 cm wide at a distance of 5 cm from the odour source, and ≈ 30 cm wide in front of the gated area. After 15 minutes the plume took over the whole tank.
** The narrow cone was ≈ 2 cm wide at a distance of 5 cm from the odour source, and was ≈ 25 cm wide in front of gate. This plume maintained its shape for the duration of the experiment.

All trials were recorded via a high-resolution overhead camera, linked to a Super VHS recorder. The fluvarium was enclosed by black plastic to shield the fish from external disturbance and to maintain the desired 16 h light: 8 h dark photoperiod; simulated daylight was established via one indirect 25 W dim-light (≈ 1 lux at the water surface). Two infrared lights (Vitek VT-IR1/12) were positioned near the camera to compensate for the darkened conditions.

Protocol

The afternoon prior to each trial, three male carp were fed, measured (TL, mm) and transferred into the fluvarium. Again, an attempt was made to match the size of individuals allocated to tanks, and fish were acclimated overnight at the desired photoperiod (16 hr light: 8 hr dark), light intensity (c. 1 lux at the water surface) and base flow (<0.1 $cm.sec^{-1}$) to maintain stable water levels and temperatures. The following morning flow conditions were adjusted sequentially, and for each trial, fish were allowed 15-min to acclimate before data recording began. However, for experiments that employed food odour, we incorporated an additional time period to ensure that the odour made it to the gated area before data recording began (under No-Flow this was 208 sec; Flow was 20 sec). The behaviour of carp within each Flow x Odour combination was recorded for15-min, after which time the flow was adjusted for the next treatment 'run'. At the end of each day, the fluvarium was drained and refilled before new fish were introduced and acclimated overnight. The reservoir tanks were also half-drained and refilled using water from an auxiliary storage tank.

Odour stimuli

To make the odour stimuli, 10 g of flaked goldfish food (Aquatronics, Tetramin® flakes) was added to 1 L of water for 1 hr. The resultant solution was filtered to remove particulate matter, and introduced to the fluvarium using a peristaltic pump at 50 ml.min^{-1}. Control odour was well-water alone.

Recorded Data

We analysed all 15-minute recordings using the same video equipment as in Experiment 1. For each recording (a 'run' within a trial), we scored the position and orientation of test fish every 10 seconds. This enabled the collation of data on three behavioural responses to each combination of current-velocity and odour:

1. Position in tank: Time spent in each test area (Gate, Left, Right, Centre). In this instance, we hoped to determine whether specific flow and odour combinations could a) 'pull' carp from their preferred habitat (inside the gated area) and b) attract them to the source of the odour.
2. Success at finding the odour source: Number of times the pipette that introduced odour into the fluvarium was touched.
3. Initial time to odour-pipette. The time it took to touch pipette for the first time. If no fish touched the pipette within the 15-min period, this was scored as 15-min.

For all recorded behaviours, a fish was deemed to be within an area if the tip of the snout was within that area.

Statistical Analysis

To answer the primary question "Are carp more efficient at finding an odour source in flowing water than in still water", we examined three sub-questions:

1. Which flow x odour combination was most successful at attracting carp from their preferred habitat?
2. Were carp most attracted to the middle section of the fluvarium, where the odour was released?
3. Did fish find the odour source more quickly in the presence of flow?

Sub-question 1 was addressed via a repeated-measures ANOVA (STATISTICA 6, StatSoft®) with Least Significant Difference (LSD) post-hoc tests in a stepwise fashion. For sub-question 2, the relative number of carp found in the central (upstream) and gated (downstream) areas were compared using a Fisher's exact test (GraphPad, Instat). All of the flow (No Flow vs. Flow) and odour conditions (No Flow + Odour vs. Flow + Odour) were compared. The controls were also compared to their matching odour (No Flow + No Odour vs. No Flow + Odour and Flow + No Odour vs. Flow + Odour). For sub-question 3, time to the odour source was compared using a Friedman Test (Nonparametric Repeated measures ANOVA, Graph Pad Instat). If there was an overall significant difference, Dunn's Multiple Comparisons Test was used to determine where the differences lay. For all analyses, statistical significance was determined at $\alpha = 0.05$.

RESULTS

Flow & odour attractants

When there was No Flow and No Odour, fish spent almost 80% of their time in the gated area. Subsequently, with the onset of flow, fish spent significantly less time in the gated area (c. 40%), even in the absence of food odour (P = <0.001). Fish also spent more time out of the gated area in the presence of odour, even when there was no measurable flow (P = <0.01). However, Flow + Odour was no better than Flow alone, in enticing carp from the preferred habitat within the gated area (P = >0.05).

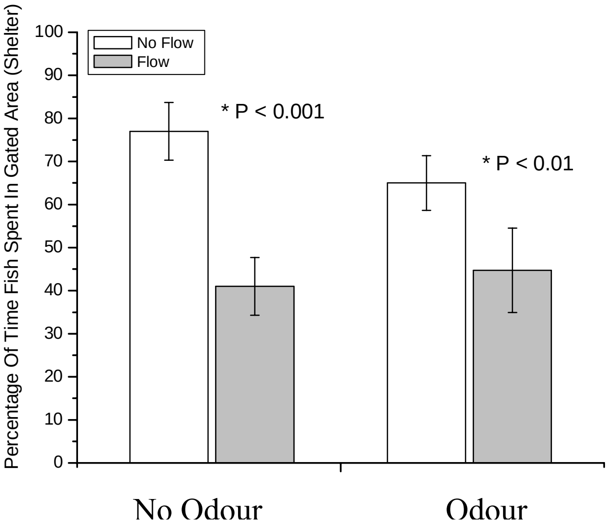

Fig 2B. Relative time spent in the gated area for each combination of flow and odour.

Position in odour plume

In the absence of flow and odour, when carp moved into the upstream area, they did so without pattern or preference for any region (No Flow x No Odour, Fig. 2C). With the onset of Odour (No Flow) and Flow (No Odour), carp spent significantly more time in the upstream section of the fluvarium. However, under these conditions, there was a significant bias toward the right-side of the fluvarium. This bias disappeared when Flow *and* Odour was introduced; odour was strongly attractive in the presence of flow, and significant numbers of carp positioned themselves within the mid-section of the fluvarium (P<0.001; Fig. 2C).

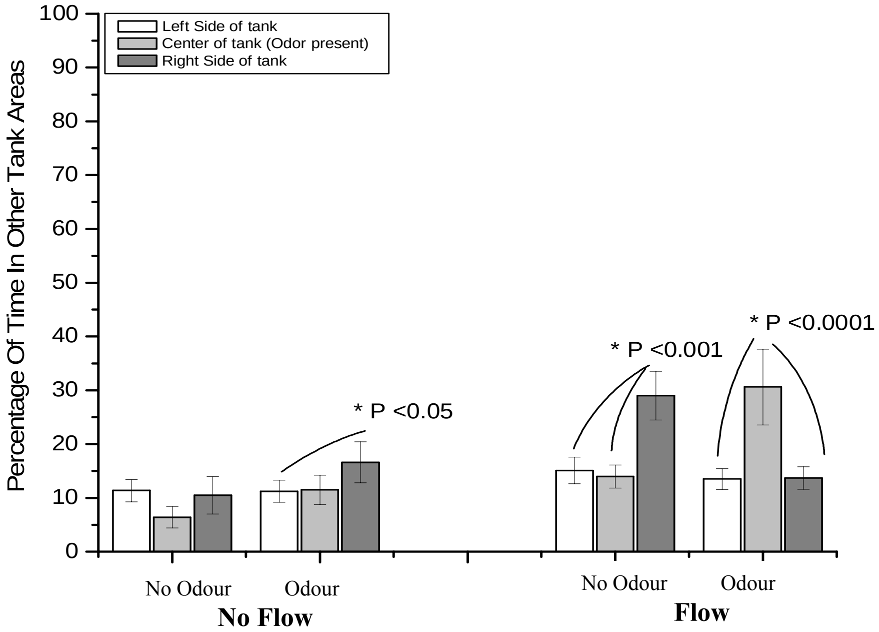

Figure 2C. Relative time spent out of the gated (shelter) area.

Success and time to odour source

In the absence of flow and odour carp virtually ignored the odour source (pipette); occasional probing occurred, but seemingly, this was without intent. In contrast, carp increasingly probed the pipette with the onset of flow (no odour), odour (no flow) and flow + odour. Indeed, under these conditions, the average percentage of carp that probed the odour source increased from 0 to 10, 15 and 31% respectively. The following comparisons were all significantly different ($\alpha = 0.05$, Table 2C):

- No Flow x No Odour vs. Flow x No Odour
- No Flow x Odour vs. Flow x Odour
- No Flow x No Odour vs. No Flow x Odour
- Flow x No Odour vs. Flow x Odour

Table 2C. Percent success and time to odour source for each flow x odour combination.

Treatment	Success (%) in finding odour source	Time to source (median, sec)	Upper quartile	Lower quartile
No Flow x No Odour	0	900	900	900
Flow x No Odour	10	900	900	900
No Flow x Odour	15	900	900	559
Flow x Odour	31	376	900	171

The time taken to reach the odour source was only significantly reduced in the presence of flow and odour (Table 2C); the *median* time to source was ≈6 min. rather than 15 min. ($p< 0.001$).

DISCUSSION

The flow velocity tested (3 $cm.s^{-1}$) was about half that determined to illicit a rheotactic response in Experiment #1. Yet, as in Experiment #1, this experiment showed that carp were positively rheotactic and would orient and swim upstream (out of the gated region), even in the absence of odour. This rheotactic response was amplified by the presence of food odour, which not only motivated fish to spend significantly greater periods of time in the middle of the fluvarium but also to investigate the odour source. In the absence of flow, carp were virtually unable to detect the odour and/or find the odour source. This might have been a consequence of the poor spatial definition of the odour plume, but indicates the important role of current-flow in the orientation and searching behaviour of carp.

GENERAL DISCUSSION

In south-eastern Australia, common carp are most abundant in shallow, warm, turbid, low-altitude wetlands that are rich in organic matter and submerged and fringing vegetation (Gehrke *et al.*, 1995; Nichols & Gilligan, 2004). For the most part, shallow wetlands are lentic (i.e., still waters) but flow, in concert with seasonal changes in temperature and photoperiod, is thought to have a significant influence on carp movement patterns (Dooland *et al.*, 2000). Indeed, carp are often the first species to enter floodplain wetlands when they fill in spring and are often the last to leave (Stuart & Jones, 2002). As such, it is tempting to ponder whether the use of flow alone, or in combination with chemical stimuli (odours and possibly pheromones), might be used to attract wetland carp into traps; for example by re-directing river flows, or by creating artificial flows using pumps.

To be effective, the abovementioned methods must be able to tempt carp from their preferred habitats, and entice them to orient and move toward the source of the current in-flow. In this study, we found that flow alone showed the potential to achieve these goals, and that flow combined with a simple food odour was especially attractive to carp; the attraction response to flow was amplified, in a synergistic manner, with the detection of odour. Further, rheotaxis and attraction occurred at flow rates as low as 3 cm sec^{-1} and these responses persisted in both fluvarium and round tanks for the entire duration of our tests. While the fish did not cluster tightly at the water- or odour-inlets, repeated approaches (attraction) were nevertheless noted. Regrettably, we did not have the opportunity to test other odour concentrations, or even different odours such as pheromones.

In apparently the only other study to address the behavioural and orientation response of carp to current-flow, (Dooland *et al.*, 2000) also found that carp demonstrated 1) rheotaxis at marginal current-flow velocities (<10 $cm.s^{-1}$), and 2) increasing attraction with increasing flow (10-50 $cm.s^{-1}$). However, in these experiments, the size of the fluvarium (80x40x50 cm deep) meant that the carp (300-400 mm TL) had no choice but to swim against the flow or risk being swept into the downstream fence. Further, no choice of flow regime (i.e. no-flow v flow) was offered.

The application of laboratory data to field situations is sometimes questioned, as the laboratory conditions may affect the volitional response of fish (Dooland *et al.*, 2000). For instance, in exposed enclosures, carp appear skittish and agitated, and prefer to aggregate in the darkest regions of those enclosures (Mallen-Cooper, 1996; Dooland *et al.*, 2000; Champion *et al.*, 2001). To compensate for the artificial conditions that we imposed on the carp, we employed dim lighting (c. 1 lux at the water surface), 360° visual screening and remote monitoring, and we ensured that the laboratory environment was as quiet as possible; we observed that the noise of a distant door closing or even heavy footsteps were enough to disturb the carp. These precautions seemed to have been successful, as rapid acclimation (within 5 minutes) to each new flow velocity or flow x odour combination was noted. Mallen-Cooper

(1996) suggests that ‘ex-situ results are not likely to overestimate the swimming ability [or flow preferences, etc.] of fish in the wild and will at best equal or underestimate their response’.

In conclusion, we have shown that carp are rheotactic at current-velocities below 35 cm.sec^{-1}, and we are optimistic that flowing water could be used alone, or in combination with a chemical stimulus, as a means to attract and capture common carp in the field. This application heralds considerable promise because both flow and odour are (relatively) easy and inexpensive to manipulate in the field. Future studies should investigate the use of different odours, including pheromones.

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