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Ecology of common bully (*Gobiomorphus cotidianus*) in the Tarawera and Rangitaiki rivers: isolation by inland distance or anthropogenic discharge?

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Abstract Previous research has identified distinct genetic, life-history and reproductive differences between populations of common bully (*Gobiomorphus cotidianus*) upstream and downstream of a pulp and paper mill outfall on the Tarawera River in the Bay of Plenty, New Zealand. This study investigated the distribution of common bully in the Tarawera River by examining fish collected from upstream (37 km inland) and downstream (20 km inland) locations and comparing them to fish from similar inland locations (40 km and 17 km inland, respectively) in the nearby Rangitaiki River. Reproductive divergence was observed between upstream and downstream sites of both rivers by differing annual trends in gonadosomatic index. Stable carbon and nitrogen isotopes confirmed residency at each sampling site and otolith microchemistry demonstrated different life-history strategies between upstream and downstream populations. Diadromous recruits dominated in both downstream river populations, with a general disappearance of diadromy upstream. A mixture of diadromous and non-diadromous fish were found in the upstream Rangitaiki River, whereas diadromous recruits were absent in the upstream Tarawera River. A reduction in oculoscapular canal structures also coincided with loss of diadromy in fish from both rivers. A behavioural study to determine whether pulp and paper mill effluent may deter fish migration within the Tarawera River demonstrated a strong avoidance of effluent, but only at concentrations (>25%) greater than those that naturally occur in the river (<15%). The results of this study suggest that combinations of influences coupled with inland distance are likely to be responsible for the isolation of common bully subpopulations within the Tarawera River.

Keywords diadromy; pulp and paper; reproduction; avoidance; otolith; stable isotopes

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INTRODUCTION

Of the seven endemic *Gobiomorphus* species in New Zealand, only the common bully (*G. cotidianus* McDowall 1975) is known to be facultatively amphidromous (McDowall 1990). Although common bully may readily form non-migratory lacustrine populations (McDowall 1990, 2000), recent evidence suggests that fluvial populations may abandon the migratory strategy where suitable larval and juvenile rearing habitats exist (Closs et al. 2003). Two recent studies (van den Heuvel et al. 2007; Michel et al. 2008) of common bully in the Tarawera River, Bay of Plenty, North Island, have documented the co-existence of genetically distinct subpopulations with opposing life histories and spawning timing within a geographically confined range. However, these studies (van den Heuvel et al. 2007; Michel et al. 2008) attributed the observed divergence of the inland Tarawera River ecotype to its separate origin from introduced Lake Tarawera stock, rather than inland distance and life-cycle river residency as might be implied by the study of Closs et al. (2003).

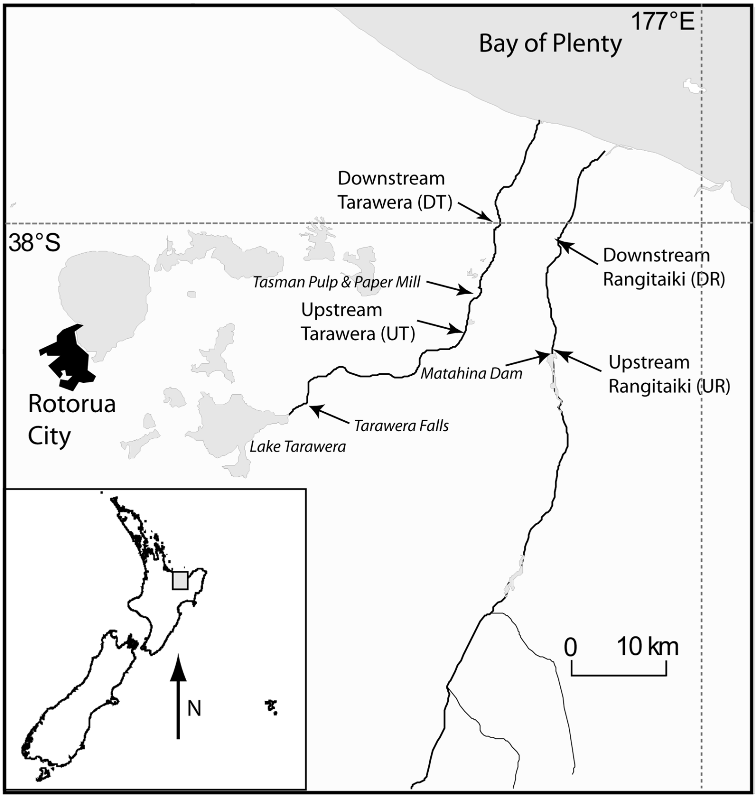

Fig. 1 Map of fish sampling locations on the Tarawera and Rangitaiki rivers, Bay of Plenty, New Zealand.

Changes in diadromous migratory behaviours may occur as the result of natural processes such as stream mouth closure or through anthropogenic barriers (McDowall & Eldon 1980; McDowall 1993; Joy & Death 2000). Anthropogenic discharges are also capable of influencing fish behaviour, potentially acting as chemical barriers through avoidance responses. Fragmentation of spawning habitat and spatial separation may result in asymmetrical gene flow, and lead to rapid divergence and reproductive isolation in some species (Hendry 2001; Gosset et al. 2006). Although no known physical barriers exist between the sampling sites of van den Heuvel et al. (2007) and Michel et al. (2008), the downstream Tarawera River was extensively modified during the 1950s through wetland drainage and channelisation, significantly altering habitat and extending river length which may have led to population divergence. The downstream Tarawera River receives significant pulp and paper mill effluent discharges (10–15% annual river flow), and has historically been subject to seasonal fluctuations in temperature and dissolved oxygen levels (Rutherford et al. 1991; Taylor & Park 2001; Landman et al. 2004, 2005).

Previous studies have shown that fish may actively avoid a variety of environmental stressors such as thermal effluents, gas supersaturation (Gray 1983), pesticides (Hogue 1999; Saglio et al. 2001), metals (Atland 1998; Scherer & McNicol 1998), chlorine (Cherry et al. 1979), suspended sediment (Boubée et al. 1997), and low pH (Kroon 2005). Thus, the presence of pulp and paper effluent represents a possible factor influencing fish distribution within the Tarawera River.

Potential barriers to upstream fish migration in the Tarawera River remain largely unexplored. The objective of the current study was to determine if a lack of diadromous recruitment to the upstream Tarawera River may be influenced by inland distance or downstream pulp and paper mill effluent effects, resulting in the observed divergence of upstream and downstream ecotypes. If solely owing to inland distance, it is hypothesised that a similar divergence of common bully ecotypes would be observed in the adjacent Rangitaiki River. We used a combination of methods to verify the life histories (diadromous versus non-diadromous) of fish at each site, and laboratory-based preference experiments were performed to examine the possibility of contaminant-induced avoidance limiting upstream fish movements within the Tarawera River.

MATERIALS AND METHODS

The study sites were the Tarawera and Rangitaiki rivers, Bay of Plenty, New Zealand (Fig. 1). The Tarawera River sites were chosen to represent an upstream, undisturbed site at the Kawerau Township (UT) and a downstream pulp mill-impacted site at Otakiri (DT). Inland river distances for these sites were 37 km and 20 km, respectively. The Rangitaiki River sites approximately matched the inland distances of the Tarawera River sites. The upstream Rangitaiki River site (UR) was 40 km inland immediately below the Matahina hydroelectric dam. The downstream Rangitaiki River site (DR) was 17 km inland between Te Teko and Edgecumbe.

Fish collections and sampling

Bimonthly common bully collections were conducted between January and December 2007 from paired upstream and downstream river sites. Approximately 20 common bully (10 male, 10 female) per site and sampling period (n = 590) were captured using minnow traps set overnight (LT, LR, UR) or by evening spotlight and dip-netting (UT). Fish were identified to species using a combination of body coloration, fin ray counts and oculoscapular canal morphology according to McDowall (2000). Fish were killed by anaesthetic overdose (0.1 g/litre MS-222, Sigma-Aldridge) before necropsy. Each fish was individually measured for total weight and somatic weight (carcass minus viscera; ± 0.1 g), total length (± 1 mm) and organ weights (liver, gonad; ± 0.01 g). Carcasses were stored frozen at –20°C pending analysis of stable isotopes, otolith microchemistry and vertebral counts.

For behavioural experiments, pulp and paper mill effluent-naïve common bully (50–65 mm total length; n = 100) were collected from Lake Tarawera using two lines of 10 minnow traps set at 1–5 m depth overnight during December 2007. Upon retrieval, fish were transferred to the laboratory in 20 litre plastic pails within 1 h. Fish were held in flow-through aquaria supplied with dechlorinated, aerated Rotorua City tap water at 15.0 ± 0.5°C. Animals were maintained on a natural photoperiod, fed daily with frozen bloodworms (Advanced Hatchery Technology Inc., Salt Lake City, United States) and held for less than 1 week before experimentation to minimise acclimatisation to artificial conditions.

Stable isotope analysis

The analysis of stable isotopes of nitrogen (N) and carbon (C) in fish tissue provides evidence of the source of nutrition but may also vary between localities because of differing water chemistry or the import of allocthanous organic material (Hicks 1997). Significant and stable differences in isotopic ratios between sites may therefore indicate long-term residency of fish. Stable isotope analyses were performed on 20 fish from each site (10 male, 10 female; n = 160) captured in May and August 2007 to establish site residency. Approximately 1 g pieces of skinless white muscle tissue were freeze-dried, ground using a mortar and pestle, and stable C and N isotopes were analysed on a fully automated Europa Scientific 20/20 stable isotope analyser according to the methods of Hicks et al. (2005) (Waikato Stable Isotope Unit, Waikato University, Hamilton, New Zealand). $\delta^{13}C$ results have a precision of ± 0.1‰ and were compared to a pre-calibrated C4-sucrose cross-referenced to Pee Dee belemnite. $\delta^{15}N$ results have a precision of ± 0.3‰ and were compared to a urea standard that is traceable to atmospheric nitrogen.

Vertebral counts

Vertebral count typically differs between diadromous and non-diadromous populations of fish with the latter typically showing a decrease in vertebral count (McDowall 2003; Ward et al. 2005). Between 13 and 35 fish from each site were fixed onto flat, non-corrugated cardboard and X-rayed (Angelsea Woman's Health, Hamilton, New Zealand) using a high-resolution mammography radiograph machine. The vertebral count of fish was individually determined from the resulting radiographs using a stereomicroscope. Counts included all vertebrae except the hypural plate.

Identification of canal morphotypes

Oculoscapular canal morphology of common bully differs between landlocked lake and riverine populations and it has been postulated that fully developed canal morphology reflects diadromous recruitment in the latter (McDowall 1990). Morphological analyses (n = 52 to 60 fish from each site) on the supraorbital section of the oculoscapular canals were performed under a stereomicroscope. Canal morphotypes were distinguished according to Michel et al. (2008) with the addition of a further canal configuration and classified into four categories.

Type 1 was characterised by paired median pores as well as anterior and posterior lateral pores; type 2 had reduced median pores and hence only anterior and posterior lateral pores present; type 3 lacked any oculoscapular pores, but possessed a continuous pattern of fine papillae ranging from the nasal to eye area; and type 4 had median pores present but lateral pores absent. Canal types were recorded individually and proportions of canal types among the four sampling locations were calculated.

Otolith microchemistry

Otolith microchemistry provides evidence of marine recruitment because of the greater strontium concentration in sea water compared with fresh water (Gillanders 2005). An enriched strontium concentration in the otolith core therefore indicates marine juvenile rearing. Otolith microchemistry was conducted on approximately 12–15 fish from each of the four sites ($n = 55$). Otoliths were removed and prepared according to the methods of Michel et al. (2008). Ratios of Sr:Ca and Ba:Ca were measured in the core and edge of each otolith by laser ablation inductively-coupled plasma mass spectrometry (LA-ICPMS, New Wave UP 213 laser ablation system and Perkin Elmer Elan SCIEX DRCII ICPMS) using helium/argon carrier gas to enhance sensitivity through increased production and transfer of ablated particles to the ICPMS (Swearer 2000; Zacherl et al. 2003). Otoliths were placed in the sample cell and the central nucleus and edge were located for spot analysis at 400× magnification with a video imaging system. Experimental conditions for the laser and ICPMS system were optimised according to the sample size. Laser spot size varied from 40–50 μm diameter, depending on otolith size, and to a depth of 5 μm with a 5 Hz repetition rate. Laser power was set at 55% output with a 40 s firing duration. After analysis of each sample, the ablation chamber was purged for 90 s with carrier gas. Additionally, the laser was fired at 0% power to standardise against interferences from the carrier gas. The elemental standard used was a NIST 612 glass (National Institute of Standards and Technology, United States) included within the sample cell.

Data from the ICPMS were analysed using the Glitter software program (Access Macquarie Ltd, Australia). Control measurements were subtracted from sample counts per second (cps) to overcome any polyatomic interference. Elemental ratios were calculated from peak cps for each otolith. Results are presented as dimensionless units for each element standardised to counts of ^{43}Ca rather than absolute concentrations, an accepted technique in the absence of matrix-matched standards (Morales-Nin et al. 2005).

Behavioural avoidance

The behavioural avoidance apparatus and protocol were modelled on those of Baker & Montgomery (2001). The experimental apparatus consisted of a main chamber (18 litres) giving fish access to two choice chambers (4 litres each) entered via funnels (OD 100 mm, ID 20 mm) which prevented return. Effluent and reference water were held in two 150-litre plastic drums and fed to the choice chambers by a peristaltic pump (Masterflex, Cole-Parmer Instrument Company, United States) at a flow rate of 23 litres/h. Rubber bungs inserted into the funnels allowed the choice chambers to be filled with the appropriate test waters. When the bungs were released, water flowed from the choice chambers into the main chamber and emptied to waste at the rear of the tank. All trials were conducted in a darkened laboratory with dim overhead lighting. Both choice chambers were covered, giving fish a double incentive to leave the main chamber owing to negative phototaxis and positive rheotaxis.

Pulp and paper mill effluent was collected from the outfall of the Tasman Mill secondary treatment system and reference Tarawera River water was obtained at Kawerau township upstream of mill inputs. Water and effluent samples were stored at 4°C and used within a week of collection.

At the beginning of each trial, the main chamber and one choice chamber were filled with reference water and the other with effluent. An individual fish was introduced into the main chamber and allowed to acclimatise for 15 min. The peristaltic pump was then switched on and the rubber bungs released to initiate current flow. Each fish was left undisturbed for 15 min after which its position was recorded. Following each trial, the apparatus was cleaned with dechlorinated tap water before the next trial.

Average effluent dilution in the Tarawera River is 12.5% (R. Donald, Environment Bay of Plenty pers. comm.). A geometric effluent dilution series (0.0, 12.5, 25.0, 50.0 and 100.0% v/v) was used to determine the behavioural preference/avoidance of effluent-naïve fish. For each concentration, 20 trials were conducted with a different set of 20 fish without reuse of any animals. The effluent chamber was alternated between each treatment to eliminate any possible left/right bias. Twenty trials at 0.0% effluent concentration (100.0% river water) were used as a control experiment to observe if common bully showed left/right bias.

Water chemistry

To compare otolith microchemistry with ambient environmental elemental composition, seasonal water samples were collected from all river sampling sites during fish collections, as well as coastal seawater samples (c. 3.2 km offshore). Seawater samples were diluted 1000-fold to avoid salt saturation of the ICPMS. River and seawater samples were individually filtered (0.45 μm) and ultrapure nitric acid (Scharlau Chemie, Spain) added to a final concentration of 2% to solubilise metals. Samples were refrigerated (4°C) and stored in the dark pending analysis. Samples were analysed for 28 elements (Li, B, Na, Mg, Al, P, K, Ca, Cr, Fe, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Ag, Cd, In, Ba, La, Hg, Tl, Pb, Bi and U) using the same ICPMS used for otolith laser ablation.

Data analysis

Descriptive statistics and statistical comparisons were calculated using STATISTICA v.8.0 software. Differences in stable isotope ratios between sites were tested for using Wilks's lambda test (Manly 2005). Female gonado-somatic index (GSI) was calculated as gonad weight/somatic weight × 100. Preference or avoidance of effluent was tested by comparing observed frequencies to expected frequencies using the Pearson chi-square test (Zar 1996). Expected frequencies were those observed in the control condition (0.0% effluent). The critical level of statistical significance for all tests was $\alpha = 0.05$.

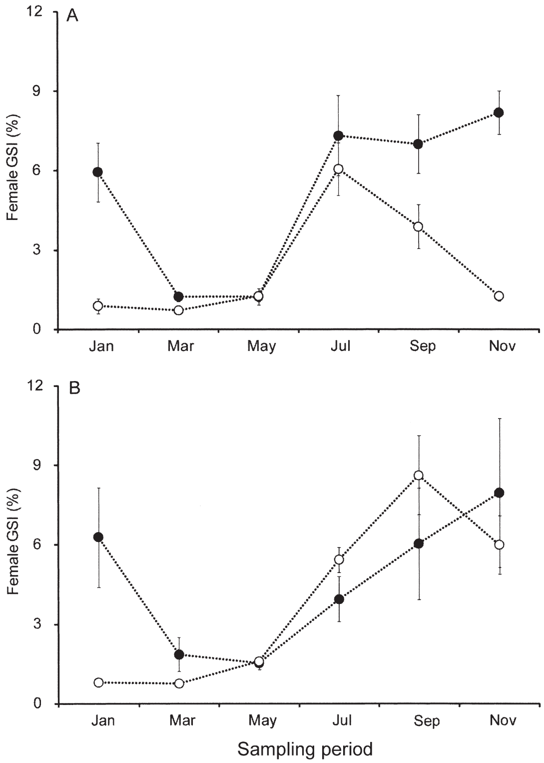

Fig. 2 Seasonal gonado-somatic indices (GSI, %; means ± SEM, $n = 7$ to 26 samples per site per period) of female common bully (*Gobiomorphus cotidianus*) sampled bimonthly from the **A**, Tarawera and **B**, Rangitaiki rivers, New Zealand, between January and November 2007. Closed symbols, upstream sites; open symbols, downstream sites.

RESULTS

Gonadal maturation commenced from May in all populations, but obvious differences in peak gonad development were observed between locations (Fig. 2). A narrow peak in GSI exhibited at both downstream river sites (DT, DR) between July and September implied discrete winter spawning in these populations. In contrast, GSI in the two upstream river populations (UT, UR) was elevated over prolonged periods, from winter (July) to midsummer (January).

Significant site differences were observed for $\delta^{13}C$ and $\delta^{15}N$ isotopes between sites (Wilks's lambda test, approx. $F = 391$, $P < 0.001$) and rivers ($F = 1203$, $P < 0.001$; Fig. 3). A trend of decreasing $\delta^{13}C$ and $\delta^{15}N$ values occurred downstream in both rivers. Rangitaiki River fish generally had markedly higher $\delta^{15}N$ values than those in the Tarawera River.

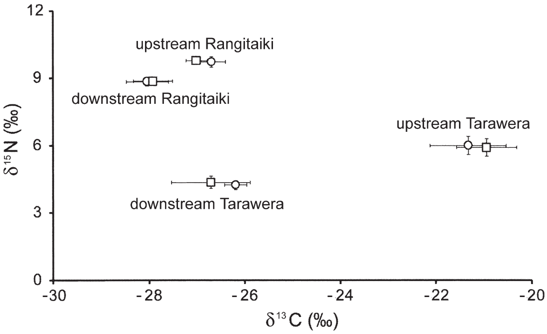

Fig. 3 Ratios of carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) from common bully (*Gobiomorphus cotidianus*) white muscle tissue. Values are means ± 95% CIs for May (circles) and August (squares) 2007 samples ($n = 20$ fish per site per period) from upstream and downstream Tarawera and upstream and downstream Rangitaiki rivers, New Zealand.

Downstream Tarawera River fish showed a markedly greater reduction in $\delta^{13}C$ (5‰) than downstream Rangitaiki River fish (1.5‰) compared with their respective upstream populations. Repeated sampling (May and August) indicated no change in isotope ratios at each site with time and the limited variation at each site implied prolonged site residency of fish.

Vertebral counts varied between 28 and 29, with most fish having 29 vertebrae (Table 1). However, DR was the only site where vertebral counts of 29 per individual were consistently observed. The UT, UR and DT sites all exhibited fish with the lower vertebral count of 28 (19%, 8%, and 13%, respectively).

High variation in oculoscapular canal morphotypes was observed between sampling sites (Table 1). Individuals with full canal formation (Type 1) were observed at all locations, but the highest proportions occurred at both downstream sites (DR 100%; DT 93%). Numerous individuals at the UT and UR sites had under-developed oculoscapular canals (Type 2, 24% and 4%, respectively; Type 4, 12% (UT)) or had lost all canals (Type 3, 25% and 65%, respectively). There was also a small proportion of DT fish with canals absent (Type 3, 5%) or under-developed (Type 4, 2%). The highest proportion of fish without canals was found in the UR (65%). The UT fish also showed various degrees of lateral canal deformities and asymmetrical pore patterns.

Water analyses and otolith microchemistry

Fourteen elements were present in water samples from fish sampling sites or coastal sea water at concentrations that exceeded ICPMS detection limits (Table 2). Similar average Ba:Ca ratios of approximately 1.7‰ were found in water samples from both rivers, although total Ca concentration was 40% greater in Tarawera River water. Highest concentrations of Sr and Ca were measured in sea water with a molar ratio of approximately 13.2‰, followed by values of 6.7 and 3.6‰ in the Rangitaiki and Tarawera rivers, respectively. The Tarawera River had elevated concentrations of geothermally-derived elements such as As, B, Hg and Li, as well as greater salinity than the Rangitaiki River.

Dimensionless ratios of Sr:Ca and Ba:Ca for individual fish otoliths showed that in fish presumed to be diadromous recruits, the Sr:Ca ratios in the core of the otolith were higher (mean + SEM: 7.00 ± 0.18‰, n = 36) than in non-diadromous individuals (2.64 ± 0.24‰, n = 24), and all exhibited a characteristic increase in Ba:Ca and decrease in

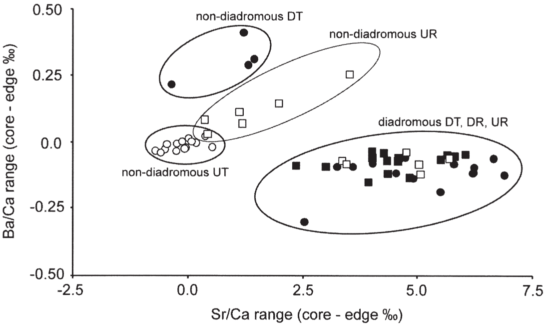

Fig. 4 Discrimination of diadromous from non-diadromous populations of common bully (*Gobiomorphus cotidianus*) from each river site (UT, upstream Tarawera, open circles; DT, downstream Tarawera, closed circles; UR, upstream Rangitaiki, open squares; DR, downstream Rangitaiki, closed squares) based on the ranges (‰) of $Sr^{88}:Ca^{43}$ and $Ba^{137}:Ca^{43}$ between otolith core and edge. (n = 12 to 18 samples per site.)

Sr:Ca from the nucleus to the edge (Fig. 4). All UT fish clustered together to exhibit a non-diadromous profile indicative of an exclusively freshwater life history, whereas up to 50% of the UR fish appeared to be diadromous (Table 1). However, UT fish did not exhibit high core or edge Ba:Ca ratios typical of freshwater resident fish and did not match Ba:Ca profiles of presumably non-diadromous Tarawera River individuals. Non-diadromous individuals with high core Ba:Ca ratios were present at both the DT (29%) and the UR (50%) sites. Fifty percent of the UR fish population showed both relatively high Sr:Ca (4–5‰) and low Ba:Ca (0.1–0.3‰) in the core. These fish were presumed to be non-diadromous considering the high natural Sr in the Rangitaiki River. The higher Sr:Ca ratio in the Rangitaiki River water matched relatively higher otolith Sr:Ca edge values in the Rangitaiki River fish (UR 2.90 ± 0.21; DR 2.59 ± 0.08) compared with the Tarawera River fish (UT 2.06 ± 0.07; DT 1.56 ± 0.09). The greatest proportion of migratory common bully (those exhibiting both high Sr:Ca and low Ba:Ca core values) occurred in the downstream sites in both rivers. All sampled DR fish and approximately 71% of DT fish appeared to be diadromous.

Fish behaviour

Fish showed a strong preference for river water when simultaneously exposed to high (50.0 or 100.0%) effluent concentrations (Fig. 5). When

Table 1 Descriptive analyses of vertebral counts, oculoscapular canal morphotypes and otoliths, for each river site. (UT, upstream Tarawera; DT, downstream Tarawera; UR, upstream Rangitaiki; DR, downstream Rangitaiki; N_V, number of samples included in vertebral analyses; %29, percentage of fish with 29 vertebrae; %28, percentage of fish with 28 vertebrae; N_M, number of samples in oculoscapular meristic analyses; type 1, 2, 3 and 4, percentages of each morphotype in sample; N_O, number of samples included in otolith analyses; %ND, percentage of non-diadromous fish; %D, percentage of diadromous fish.)

	Vertebral counts			Oculoscapular canal morphotypes					Otoliths		
Site	N_V	%29	%28	N_M	Type 1	Type 2	Type 3	Type 4	N_O	%ND	%D
UT	21	81	19	57	39	24	25	12	14	100	0
DT	24	87	13	60	93	0	5	2	14	29	71
UR	13	92	8	52	31	4	65	0	12	50	50
DR	35	100	0	58	100	0	0	0	15	0	100

Table 2 Mean (± SEM) water elemental chemistry of river sites (UT, upstream Tarawera; DT, downstream Tarawera; UR, upstream Rangitaiki; DR, downstream Rangitaiki) and coastal seawater samples. Sea water detection limits are high owing to sample dilution required for inductively-couple plasma mass spectrometry (ICPMS) analysis (BDL, below detection limit. The following elements were below detection limit in all samples: Al, Co, Ni, Cu, Zn, Se, Ag, Cd, In, La, Tl, Pb, Bi, U).

	ICPMS detection limits		Site				
Element	Fresh water (μM)	Sea water (mM)	UT (μM) (n = 5)	DT (μM) (n = 5)	UR (μM) (n = 5)	DR (μM) (n = 5)	Sea water (mM) (n = 3)
Li	0.03	0.03	40.5 (1.1)	44.2 (1.2)	1.36 (0.04)	1.29 (0.07)	BDL
B	0.46	0.46	29.0 (0.8)	64.2 (1.8)	6.84 (0.38)	6.43 (0.48)	BDL
Na	0.87	0.87	2040 (62)	2600 (88)	366 (16)	342 (23)	391 (22)
Mg	0.82	0.82	240 (11)	229 (9)	85.6 (3.6)	81.1 (5.5)	51.7 (3.6)
P	0.65	0.65	1.91 (0.34)	2.71 (0.26)	0.67 (0.16)	0.83 (0.13)	BDL
K	1.28	1.28	134 (5)	161 (4)	65.1 (2.2)	61.2 (3.1)	8.51 (0.62)
Ca	1.25	1.25	95.5 (4.0)	123 (5)	78.4 (2.9)	76.2 (3.8)	6.12 (0.44)
Cr	0.01	0.01	BDL	0.04 (0.01)	BDL	BDL	BDL
Fe	0.36	0.36	0.913 (0.122)	1.95 (0.14)	0.63 (0.05)	2.45 (1.15)	BDL
Mn	0.009	0.009	0.226 (0.123)	0.220 (0.143)	0.032 (0.014)	0.260 (0.120)	BDL
As	0.013	0.013	0.292 (0.013)	0.418 (0.018)	BDL	0.008 (<0.001)	BDL
Sr	0.006	0.006	0.367 (0.013)	0.419 (0.011)	0.523 (0.016)	0.508 (0.019)	0.081 (0.007)
Sr:Ca (‰)			**3.84 (0.03)**	**3.40 (0.06)**	**6.67 (0.09)**	**6.67 (0.12)**	**13.24 (0.32)**
Ba	0.004	0.004	0.170 (0.011)	0.220 (0.006)	0.130 (0.006)	0.130 (0.005)	BDL
Ba:Ca (‰)			**1.81 (0.19)**	**1.79 (0.07)**	**1.66 (0.03)**	**1.71 (0.04)**	–
Hg	0.001	0.001	0.003 (0.001)	0.013 (0.002)	BDL	BDL	BDL

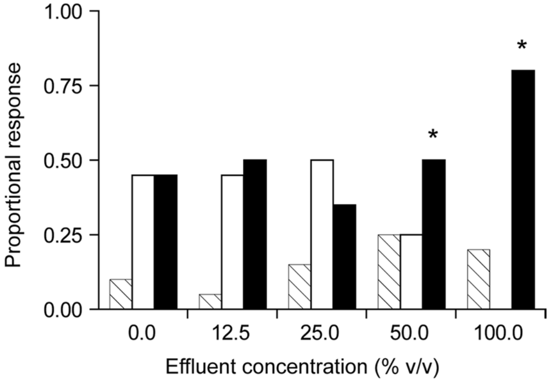

Fig. 5 Behavioural preference/avoidance responses of common bully (*Gobiomorphus cotidianus*) simultaneously exposed to Tarawera River water and 12.5, 25.0, 50.0 or 100.0% v/v Tasman Mill effluent. Data are presented as proportion of fish ($n = 20$ per concentration) that showed no response (diagonal hatching) or selected either river water (open bars) or effluent (closed bars). Significant preference for river water (*) was exhibited during 50.0 ($\chi^2 = 6.4$, $P<0.05$) and 100.0% ($\chi^2 = 16.4$, $P<0.001$) effluent exposures.

given a choice between 100.0% effluent and river water, 16 of 20 fish made a choice and, of those 16 fish, 100% avoided the effluent by moving into the control chamber containing river water ($\chi^2 = 16.4$, $P < 0.001$). Of the 15 fish that made a choice when exposed to 50.0% effluent, 67% avoided the effluent ($\chi^2 = 6.4$, $P < 0.05$). At effluent concentrations below 50.0%, there were no discernable preferences for either river water or effluent, and thus no avoidance response was inferred. No significant left/right bias was observed in control exposures.

DISCUSSION

This study demonstrated site residency, loss of diadromy and morphological changes with inland distance in sympatric populations of common bully at upstream river sites. Differences between UR and UT populations suggest that a number of factors may have influenced the ecology of common bully within these rivers. Behavioural experiments demonstrated effluent avoidance which may implicate current or historical pulp and paper mill discharges as having significant consequences for fish migration in the Tarawera River.

Stable C and N isotopes demonstrated distinctive ecosystem and catchment influences. Fish from the DT site possessed a markedly depleted $\delta^{13}C$ signature compared with fish upstream; a characteristic of pulp mill wastewater-receiving environments (Wayland & Hobson 2001; West 2007; Landman et al. 2008). The high $\delta^{13}C$ values of UT fish reflected the influence of old water from Lake Tarawera. A general downstream trend of decreasing $\delta^{13}C$ was observed in both rivers as upstream waters were diluted by tributary inputs of biogenically-derived carbon (Fitzgerald 1996). The elevated $\delta^{15}N$ values in Rangitaiki River fish were presumably linked to agricultural influences in the catchment.

Both downstream populations showed evidence of narrowly defined, winter spawning, but the upstream populations exhibited more prolonged gonadal maturation occurring from late winter throughout summer. Similar multiple or protracted spawning has been observed in landlocked common bully (Stephens 1982). Non-diadromous, landlocked common smelt (*Retropinna retropinna*) have also been found to spawn at different times compared with diadromous populations (Ward et al. 2005). Thus, an apparent relationship between spawning season and migratory behaviour suggests that shifts in reproductive timing may result from adaptive divergence (Hendry 2001) or depend upon seasonal food availability and predation in fluvial and marine systems (Ovenden & White 1990; Michel et al. 2008).

Otolith microchemistry confirmed the predominance of diadromy in downstream populations and a general loss of diadromy with inland distance. The presence of some non-diadromous fish in the DT population may be related to improved habitat and productivity as common bully may voluntarily abandon the migratory phase where suitable rearing habitat exists (Closs et al. 2003). Similarly, habitat and inland distance may explain the predominance of non-diadromous recruits upstream, although this assumption is confounded by the possibility of non-diadromous recruitment from the Matahina Dam (immediately upstream of UR) and Lake Tarawera (26 km upstream of UT). Common bully vertebral counts in both rivers were within the range (i.e., 28–29 vertebrae) previously reported for this species (McDowall & Stevens 2007). However, the only site where all fish possessed 29 vertebrae was in the completely diadromous DR population. Reduced vertebral counts were only found in populations with non-diadromous recruits, as has been observed for other New Zealand fish species (McDowall 2003; Ward et al. 2005).

Also associated with loss of diadromy were poorly developed oculoscapular canal structures in the UR and UT populations. Gobiid oculoscapular canal structures may be hereditary or environmentally influenced (Coombs et al. 1992; Ahnelt et al. 2004). Canal development is generally believed to occur during the larval and juvenile life stages (Fuiman et al. 2004; Modgans 2005), which corresponds with upstream migrations of common bully at 2–3 months post-hatching (McDowall 1990). Accordingly, Michel et al. (2008) observed no fish with full canal development in the non-diadromous UT population, although in the current study, nearly 40% of UT fish possessed fully developed canal structures. Reductions in oculoscapular canal development are observed in many non-diadromous lake populations of common bully (McDowall 1990) and in fluvial and lacustrine populations of non-diadromous *Gobiomorphus* species closely-related to common bully, including Cran's bully (*G. basalis*) and the Tarndale bully (*G. alpinus*; McDowall 2000).

Population life histories of Tarawera River sites closely matched those recorded by Michel et al. (2008). However, we did not observe any non-diadromous fish in the lower Rangitaiki River compared with the 30% reported by Michel et al. (2008). These authors speculated that genetic differences in the upstream Tarawera population were owing to dominant recruitment by upstream Lake Tarawera fish and an absence of diadromous recruitment owing to inland distance. However, Rangitaiki River data from the present study indicate that diadromous common bully may recruit at least 40 km inland, and the absence of diadromous recruits in the UT suggests that factors additional to inland distance influence fish migration in the Tarawera River system. The Tarawera River receives natural (geothermal) and anthropogenic (pulp mill and municipal effluents) inputs between the upstream and downstream sites. Although there are few studies examining the behavioural effects of modern, improved mill effluents, a number of earlier studies have shown avoidance responses to pulp mill effluents in the laboratory and *in situ* (Kelso 1977; McGreer & Vigers 1983; Birtwell & Kruzynski 1989). The current study demonstrated the ability of effluent to induce behavioural avoidance, although only at concentrations (>25% v/v) above those that naturally occur in the river (<15% v/v).

The preference for river water over effluent does not fully explain the apparent absence of upstream DT to UT migration given that fish are resident at the effluent-exposed DT site. One possibility is that pre-exposure may decrease contaminant responses through sensory adaptation or habituation. Myllyvirta & Vuorinen (1989) have shown that while effluent-naïve vendace (*Coregonus albula*) avoid a pulp and paper mill effluent, 1-week pre-exposure can result in a preference for effluent-contaminated water. A second possibility is that altered migratory phenomena may be a legacy issue linked to historical contaminant exposures.

In addition to pulp mill contaminants, elevated concentrations of geothermally-derived elements such as As, B, Hg and Li were measured in the DT water. Significant increases in these elements between the UT and DT sampling sites presumably originate from a local geothermal bore field and the release of Na from the chemical pulping process used at the pulp and paper mill. Previous studies have demonstrated that exposure to metal contaminants can induce avoidance reactions and influence upstream movements of migratory species. Sprague et al. (1965) and Saunders & Sprague (1967) found reduced upstream migrations of Atlantic salmon (*Salmo salar*) in a stream contaminated with Cu and Zn. Hartwell et al. (1987) also observed avoidance of fathead minnows (*Pimephales promelas*) to metal mixtures (Cu, Cr, and Se) and common bully have been shown to avoid Cu (Richardson et al. 2001) although only at concentrations considerably exceeding those of downstream Tarawera River water. Although common bully showed a strong avoidance response to high concentrations of pulp mill effluent, the question of whether behavioural avoidance, owing to river inputs of pulp mill or geothermal effluents, is the primary mechanism preventing upstream recruitment of diadromous common bully in the Tarawera River remains unresolved.

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