

Parasite manipulation of brain monoamines in California killifish (*Fundulus parvipinnis*) by the trematode *Euhaplorchis californiensis*

J. C. Shaw^{1,*}, W. J. Korzan², R. E. Carpenter^{3,4}, A. M. Kuris¹,
K. D. Lafferty⁵, C. H. Summers^{3,4} and Ø. Øverli⁶

¹Department of Ecology, Evolution and Marine Biology, University of California Santa Barbara, Santa Barbara, CA 93106-7130, USA

²Department of Cell Biology, Harvard Medical School, Brookline, MA 02215, USA

³Department of Biology, University of South Dakota, Vermillion, SD 57069, USA

⁴Neuroscience Group, Division of Basic Biomedical Sciences, University of South Dakota, Vermillion, SD 57069, USA

⁵US Geological Survey, Western Ecological Research Center, University of California Santa Barbara, Santa Barbara, CA 93106, USA

⁶Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, N-1432 Aas, Norway

California killifish (*Fundulus parvipinnis*) infected with the brain-encysting trematode *Euhaplorchis californiensis* display conspicuous swimming behaviours rendering them more susceptible to predation by avian final hosts. Heavily infected killifish grow and reproduce normally, despite having thousands of cysts inside their braincases. This suggests that *E. californiensis* affects only specific locomotory behaviours. We hypothesised that changes in the serotonin and dopamine metabolism, essential for controlling locomotion and arousal may underlie this behaviour modification. We employed micropunch dissection and HPLC to analyse monoamine and monoamine metabolite concentrations in the brain regions of uninfected and experimentally infected fish. The parasites exerted density-dependent changes in monoaminergic activity distinct from those exhibited by fish subjected to stress. Specifically, *E. californiensis* inhibited a normally occurring, stress-induced elevation of serotonergic metabolism in the raphe nuclei. This effect was particularly evident in the experimentally infected fish, whose low-density infections were concentrated on the brainstem. Furthermore, high *E. californiensis* density was associated with increased dopaminergic activity in the hypothalamus and decreased serotonergic activity in the hippocampus. In conclusion, the altered monoaminergic metabolism may explain behavioural differences leading to increased predation of the infected killifish by their final host predators.

Keywords: parasites; fish; dopamine; serotonin; monoamine; trematode

1. INTRODUCTION

Certain parasites manipulate their host's behaviour in dramatic ways. Typically, altered behaviours appear to increase parasite fitness by enhancing the likelihood of transmission between hosts in the parasite's life cycle (Holmes & Bethel 1972; Barber *et al.* 2000; Combes 2001; Moore 2002; Kuris 2003; reviews in Thomas *et al.* 2005). Predator hosts often consume a higher proportion of infected prey—whose manipulated behaviours render them easier to catch—a phenomenon termed parasite-increased trophic transmission (PITT; Carney 1969; Holmes & Bethel 1972; Brassard *et al.* 1982; Hoogenboom & Dijkstra 1987; Lafferty 1992; Lafferty & Morris 1996; Bakker *et al.* 1997; Thomas & Poulin 1998; Lafferty 1999; McCurdy *et al.* 1999; Berdoy *et al.* 2000; Knudsen *et al.* 2001). From both a proximate and an ultimate perspective, it remains of considerable interest whether the behavioural changes of the infected prey

result from the side effects of general debilitation, or whether the parasites actively target the host's neuroendocrine systems (reviewed in Thomas *et al.* 2005).

Recent studies have revealed possible neurobiological mechanisms of behaviour modification by parasites (Kavaliers *et al.* 1999; Thomas *et al.* 2005). The manipulation of invertebrate hosts involves parasite-secreted molecules (Beckage 1993; de Jong-Brink *et al.* 2001; Biron *et al.* 2005), and neuroanatomical and monoaminergic disruption (Adamo & Shoemaker 2000; Helluy & Thomas 2003; Thomas *et al.* 2003; Rojas & Ojeda 2005; Tain *et al.* 2006), including reduced activity of monoaminergic neurons (Rosenberg *et al.* 2006). There are fewer studies on vertebrates (see Klein (2003) for a review). Rodents infected with *Toxoplasma gondii* show increased exploratory behaviour and an attraction to cat odours, which could greatly increase *T. gondii* transmission to its feline final host (Berdoy *et al.* 2000; Vyas *et al.* 2007). *T. gondii*-infected mice display increased brain dopamine (DA) levels (Stibbs 1985), and administration of a specific DA receptor agonist can induce similar behaviours in uninfected mice (Skalova *et al.* 2006). Stickleback fishes infected with larval tapeworms (*Schistocephalus solidus*)

* Author for correspondence (shaw@lifesci.ucsb.edu).

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2008.1597> or via <http://journals.royalsociety.org>.

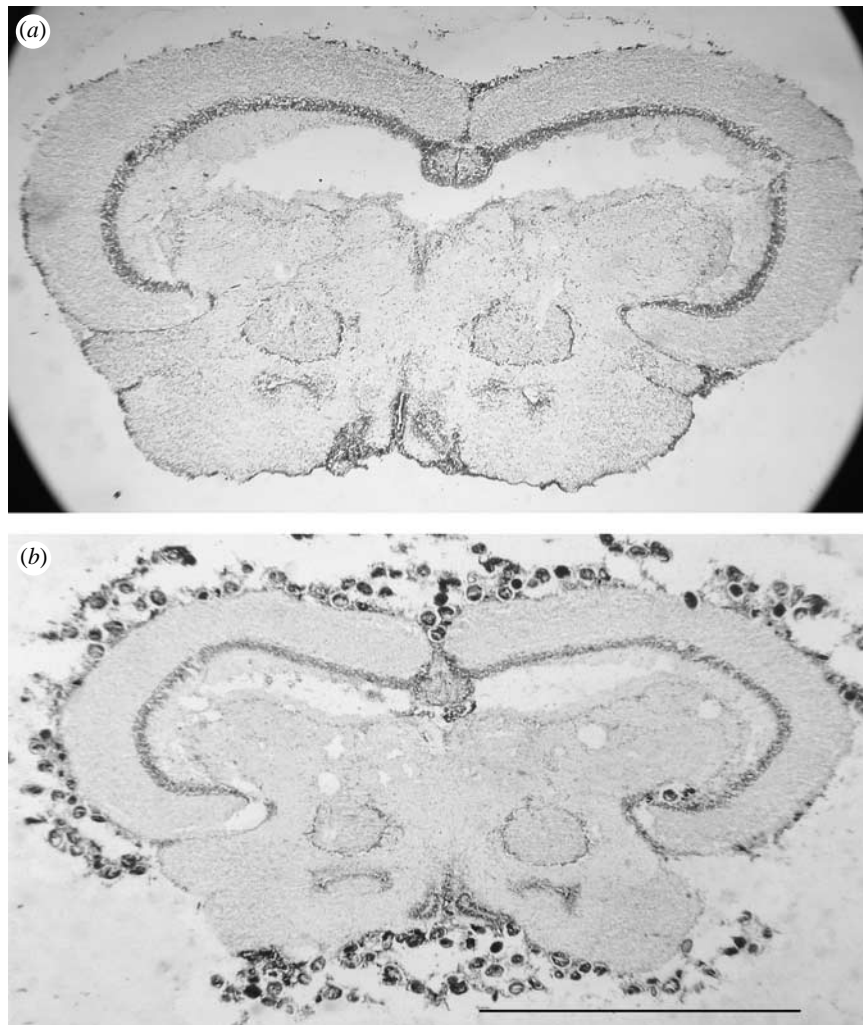


Figure 1. Cross sections through the midbrains (diencephalons) of killifish both (a) uninfected and (b) infected with *E. californiensis* (metacercariae surrounding the outer surface).

show increased serotonin metabolism in their hypothalamus and brainstem (Øverli *et al.* 2001). However, *S. solidus* infections can be quite debilitating, and the neuroendocrine responses of infected fishes are consistent with those seen in chronically stressed fishes (Winberg & Nilsson 1993).

In the present paper, we explore the potential mechanisms a trematode uses to manipulate the behaviour of the California killifish, *Fundulus parvipinnis*. This classic example of PITT involves *F. parvipinnis* and its brain parasite, *Euhaplorchis californiensis* (Lafferty & Morris 1996). The California killifish is one of the most common fishes in Southern California and Baja California estuaries (West & Zedler 2000; Allen *et al.* 2006). Infected populations occur where the California horn snail, *Cerithidea californica*, is also present in the estuary. The trematode *E. californiensis*, one of the most abundant trematode parasites in these habitats (Martin 1955; Hechinger *et al.* 2007), uses three hosts in its life cycle: horn snails (*C. californica*); killifish; and several species of piscivorous birds (Martin 1950). Larval *E. californiensis* cercariae swim out of their first intermediate snail host, penetrate the skin of *F. parvipinnis* and migrate to the brain, presumably following blood vessels or nerve tracts (McNeff 1978; Hendrickson 1979; Haas *et al.* 2007). Once inside the braincase, cercariae encyst on the pial surface of the brain as metacercariae. Infected killifish

display four times as many conspicuous swimming behaviours as uninfected ones, rendering them 10–30 times more likely to be eaten by a bird, the parasite's final host (Lafferty & Morris 1996). Prevalence of *E. californiensis* reaches 100 per cent in most localities, such that all killifish over a minimum size are infected (Shaw 2007). Although this infection pattern prevents a comparison of infected and uninfected individuals from the same population, the killifish–*E. californiensis* system provides an ideal model to investigate the specific neurobiological mechanisms of behaviour modification. Infected killifish appear otherwise healthy and school normally, despite hundreds to thousands of cysts packed around their brains (figure 1). Specific neurochemical alteration provides a plausible hypothesis for the modification of killifish behaviour by *E. californiensis* when considering the lack of gross pathology and the parasite's direct contact with the brain surface.

Monoamine neurotransmitters include the indoleamine serotonin and the catecholamines DA, adrenaline and noradrenaline and play an important role in shaping vertebrate social, locomotory and reproductive behaviours (Winberg & Nilsson 1993; Fabre-Nys 1998; Summers & Winberg 2006). These substances are considered neuro-modulators, because they have long-term neuronal effects in addition to their brief synaptic actions (Adamo 2002; Libersat & Pflueger 2004). DA and serotonin

(5-hydroxytryptamine, 5-HT) in particular influence fish social behaviour and swimming activity (Winberg & Nilsson 1993; Mok & Munro 1998; Höglund *et al.* 2001; Clements & Schreck 2004; Øverli *et al.* 2004). Considering that monoamines affect such fundamental behaviours, it is no surprise these neuromodulators respond quickly to external stimuli. Physical and social stressors can rapidly affect monoamine levels and neurotransmission in teleosts and other non-mammalian vertebrates (Winberg & Nilsson 1993; Winberg *et al.* 1993; Øverli *et al.* 1999; Höglund *et al.* 2001; Summers & Winberg 2006; Watt *et al.* 2007). Consequently, investigations of brain monoaminergic activity must be interpreted in the context of an organism's stress levels during the experiment and sampling.

Here, we examine whether *E. californiensis* infections are associated with changes in brain monoaminergic activity in experimentally infected killifish. We conducted experimental infections to (i) control for possible population differences between uninfected and naturally infected fish (which do not co-occur naturally), and (ii) isolate the effects of *E. californiensis*, as naturally infected killifish are usually infected with multiple trematode species (Yoshino 1971; Shaw *et al.* 2005). We also implemented a stress treatment in order to differentiate parasite effects from those of generalized stressors, since parasitic infection can cause chronic stress in certain hosts (Barber *et al.* 2000).

2. MATERIAL AND METHODS

(a) Animal care and experimental infection

We used minnow traps and seines to collect adult uninfected *F. parvipinnis* (mean TL=62.7 mm, s.d.=6.3) from the University of California, Santa Barbara (UCSB) campus lagoon (34°24'32" N, 119°50'50" W) in May and June 2005. Fish were housed in 38 l seawater tanks (16–18°C) at UCSB and fed commercial flakes once a day. Tank water was changed daily with aged seawater until fish were killed 2 months later. We experimentally infected half of the uninfected fish with *E. californiensis* cercariae. Briefly, cercariae were stimulated to shed from *C. californica* by holding snails dry and dark for at least 1 week, followed by immersion in seawater for 2–3 h under an incandescent lamp. Cercariae were then collected, pooled and quantified following procedures described in Sandland & Goater (2000). Fish were exposed to cercariae or sham-exposed to seawater in buckets with 8 l seawater for 2 h. Although *E. californiensis* metacercariae are infective to definitive hosts after 2 weeks, we allowed 4 weeks for infections to mature before sampling (Martin 1950). All animal care and research was conducted in accordance to policies of the UCSB Institutional Animal Care and Use Committee.

(b) Stress treatment and sampling

The experimental set-up consisted of a blind design that pooled uninfected and experimentally infected fish together in tanks containing four individuals each. *E. californiensis* infections are not visible externally and cannot be transmitted horizontally. To sample non-stress tanks, we netted all fish simultaneously and killed them immediately in MS-222 (5 g l⁻¹). Fish heads were removed immediately, frozen on dry ice and stored at -70°C. We recorded the body mass, gonad and liver mass and drew blood samples from the caudal

vein using EDTA-coated syringes. For stress treatment tanks, the water levels were lowered to a dorsal fin height (approx. 1 cm) 1 h before sampling. Each fish was netted and held in the air for 5 s, every 15 min for 1 h, after which fish were killed as described above. For the plasma cortisol, we centrifuged blood samples at 10 000 r.p.m. for 5 min at 4°C, separated the plasma and stored it at -70°C.

(c) Micropunch dissection

We chose the following brain regions for analysis based on the behavioural and neuroendocrine significance, stress response and putative homologies to mammalian systems (Northcutt 1983; Parent 1983; Winberg *et al.* 1993; Salas *et al.* 2003; Wullimann & Mueller 2004; Butler & Hodos 2005): hippocampus (dorsal lateral area dorsalis telencephali, Dl); striatum (central and dorsal areas ventralis telencephali, Vc, Vd); hypothalamus (preoptic and paraventricular nuclei, NPO and NPP); and raphe nuclei (inferior and superior, IR and SR; abbreviations from Peter *et al.* 1975 and Wullimann *et al.* 1996). Frozen whole heads were sectioned at 300 µm on a cryostat at -20°C. Brain regions were identified with stereotaxic atlases (Peter *et al.* 1975; Wullimann *et al.* 1996; Anken & Bourrat 1998) and microdissected with a 300 µm diameter punch (*sensu* Renner & Luine 1984). Microdissection did not dislocate metacercariae, which occurred on the outer surface of the brains. We quantified *E. californiensis* intensity from brain sections after microdissection.

(d) Monoamine and plasma hormone analyses

We measured DA, its metabolite DOPAC (3,4-dihydroxy phenylacetic acid), serotonin (5-HT), and its metabolite 5-HIAA (5-hydroxyindoleacetic acid) using high-performance liquid chromatography (HPLC) with electrochemical detection, as described by Korzan *et al.* (2000). See the electronic supplementary material, §1 for HPLC details. To assess the protein content for each sample, we dissolved the tissue pellet in 100 µl of cold NaOH buffer (0.8 g NaOH pellets, 50 ml deionized H₂O) and analysed each sample using the Bradford method. We assessed cortisol using a direct enzyme-linked immunosorbent assay kit from Assay Designs, Inc. Details are provided in the electronic supplementary material, §2.

(e) Statistical analysis

Unequal sample sizes, resulting from mortalities and HPLC sample loss (table 1), prevented the use of groupwise comparisons. Instead, we analysed brain monoaminergic activity and plasma cortisol using general linear models (GLMs) and regression. We used the ratio of monoamine metabolite/monoamine as an index of monoaminergic activity (Shannon *et al.* 1986; James *et al.* 1989). Data were log (5-HIAA/5-HT in hippocampus and hypothalamus) or logit transformed (DOPAC/DA in striatum) as needed to achieve normality of residuals. Model parameters included stress treatment (non-stressed or stressed) and sex as factors, metacercarial density, fish body mass and condition factor (K, ((wet body mass/total length³) × 10⁵)) as covariates, and all first-order interactions. We used parasite density (number of metacercariae/fish body mass) as the independent variable to analyse parasite effects on physiological variables, since parasite numbers tend to scale with killifish body mass (Shaw 2007). We ran an additional regression analysis when metacercarial density was a significant factor, in order to assess the density dependence; regressions were examined separately within each stress treatment group. To identify

Table 1. Sample size and morphometric data for experimental groups. Gonadosomatic (GSI) and HSI indices given as mean \pm 1 s.d., with the exception of the single experimentally infected, non-stressed female. F, females; M, males; N, sample size.

experimental group	sex	N	body mass (g)	GSI	HSI
uninfected, non-stressed	F	2	9.7 \pm 5.1	16.2 \pm 2.0	2.3 \pm 0.4
	M	15	9.1 \pm 2.3	2.4 \pm 0.4	2.5 \pm 0.6
uninfected, stressed	F	4	10.3 \pm 3.0	18.6 \pm 4.2	2.3 \pm 0.7
	M	9	9.0 \pm 2.2	2.4 \pm 0.3	2.3 \pm 0.7
experimentally infected, non-stressed	F	1	10.4	8.7	2.8
	M	2	8.6 \pm 1.6	2.6 \pm 0.1	2.8 \pm 0.4
experimentally infected, stressed	F	3	9.1 \pm 1.8	24.1 \pm 5.0	3.2 \pm 0.5
	M	2	7.8 \pm 3.2	1.8 \pm 0.5	3.2 \pm 0.5

Table 2. GLM results for serotonin (5-HT) activity, measured as 5-HIAA/5-HT, in uninfected and experimentally infected killifish. (Brain region codes defined in §2. Parasite effect indicates the effect of increasing metacercarial density on 5-HT activity. Arrows indicate increased or decreased 5-HT activity, EI, experimentally infected; K, body condition. An asterisk (*) denotes significance after correction for multiple comparisons using FDR. D.f. = 1 for all main effects and interactions.)

serotonin activity	SS	F	<i>p</i> -value	parasite effect	stress effect
hippocampus (DI) overall model: SS=0.374, $F_{5,36}=6.6$, $p=0.0003$, $R^2=0.5$					
metacercarial density	0.150	13.3	0.0010*	note interaction	
stress	0.077	6.8	0.0100*		
sex	0.021	1.9	0.1820		
metacercarial density \times stress	0.173	15.4	0.0005*	\downarrow in non-stressed fish	
stress \times sex	0.115	10.2	0.0032*		\uparrow in males
striatum (Vc, Vd) overall model: SS=0.076, $F_{4,37}=4.1$, $p=0.008$, $R^2=0.3$					
stress	0.045	9.6	0.004*		note interaction
sex	0.027	5.9	0.020*		
K	0.009	1.9	0.180		
stress \times K	0.036	7.8	0.009*		\uparrow with \uparrow K
hypothalamus (NPO, NPP) overall model: SS=0.040, $F_{1,35}=18.3$, $p=0.0001$, $R^2=0.4$					
stress	0.040	18.3	0.0001*		\uparrow overall
raphe (IR, SR) overall model: SS=0.540, $F_{4,35}=36.2$, $p<0.0001$, $R^2=0.8$					
metacercarial density	0.080	21.6	<0.0001 *	\downarrow overall	
stress	0.510	136.6	<0.0001 *		note interaction
body mass	0.016	4.4	0.0448*		
stress \times body mass	0.016	4.4	0.0436*		\uparrow in large fish

possible health differences resulting from experimental infections, we compared gonadosomatic (GSI; ((gonad mass/total length) \times 100)) and hepatosomatic (HSI, ((liver mass/total length) \times 100)) indices between infection groups separately for each sex, using GLMs with metacercarial density as a factor. For all models, we sequentially removed non-significant main effects ($p>0.1$) and interactions ($p>0.05$), conducted *post hoc* analyses using Tukey's HSD and corrected for multiple comparisons using the false discovery rate (Benjamini & Hochberg 1995). We performed all analyses using JMP 6.0, SAS Institute Inc.

3. RESULTS

Parasite density-dependent changes in monoaminergic activity occurred in the hippocampus and raphe nuclei (tables 2 and 3). In particular, *E. californiensis* density was associated with increased dopaminergic activity in the raphe nuclei and decreased serotonergic activity in the hippocampus and raphe nuclei. We present DA and 5-HT data in uninfected and experimentally infected fish, for each brain region below, including only statistically

significant factors and interactions (see tables 2 and 3 for all statistics). Experimental infections resulted in much lower parasite intensities than those found in natural infections (Shaw 2007), yet these infections still produced density-dependent changes in raphe serotonergic activity. The infection treatment did not cause significant changes in GSI or HSI in males or females (table 1). We present mean monoaminergic activity by brain region, for uninfected and experimentally infected fish in the electronic supplementary material, §4.

(a) Hippocampus

The parasite effect in the suggested hippocampus homologue (DI) varied according to stress treatment. Serotonergic activity decreased with increasing metacercarial density in non-stressed fish only, whereas there was no significant change observed in stressed fish (metacercarial density \times stress; table 2). There was no significant relationship between metacercarial density and hippocampal serotonergic activity ($p=0.1$, 0.9 for unstressed and stressed groups, respectively). The stress treatment,

Table 3. GLM results for DA activity, measured as DOPAC/DA, in uninfected and experimentally infected killifish. (Brain region codes defined in § 2. Parasite effect indicates the effect of increasing metacercarial density on DA activity. Arrows indicate increased or decreased DA activity; EI, experimentally infected fish; K, body condition. An asterisk (*) denotes significance after correction for multiple comparisons using FDR. D.f. = 1 for all main effects and interactions.)

dopamine activity					
	SS	F	<i>p</i> -value	parasite effect	stress effect
striatum (Vc, Vd) overall model: SS=4.687, $F_{6,37}=4.3$, $p=0.003$, $R^2=0.5$					
stress	1.389	7.7	0.009*		note interaction
sex	0.597	3.3	0.080		
body mass	0.132	0.7	0.400		
K	0.004	0.02	0.900		
stress×K	2.004	11.1	0.002*		↑ with ↑ K
sex×body mass	0.821	4.6	0.041		↑ in males
hypothalamus (NPO, NPP) overall model: SS=0.646, $F_{6,36}=4.1$, $p=0.004$, $R^2=0.5$					
stress	0.0004	0.02	0.900		note interaction
sex	0.092	3.4	0.070		
body mass	0.058	2.2	0.150		
K	0.014	0.5	0.470		
stress×sex	0.220	8.4	0.007*		↑ in males
body mass×K	0.123	4.7	0.040		
raphe (IR, SR) overall model: SS=0.046, $F_{5,36}=3.9$, $p=0.007$, $R^2=0.4$					
metacercarial density	0.010	4.5	0.04*	↑ overall	
stress	0.018	7.6	0.01*		note interaction
sex	0.000	0.0	0.93		
body mass	0.018	7.5	0.01*		
stress×sex	0.018	7.6	0.01*		↓ in females

by contrast, increased serotonergic activity in males but not females (stress×sex; table 2). DA activity was not measurable in this brain region owing to poor separation and low concentrations of the DA metabolite DOPAC.

(b) Striatum

We did not observe significant parasite effects on monoaminergic activity in this brain region. However, stress and sex significantly affected striatal serotonergic activity. The effect of stress also varied with body condition, so that striatal serotonergic activity was greater in the stressed fish with better condition, whereas non-stressed fish showed no significant change (stress×K; table 2). Stress and sex also affected dopaminergic activity in the suggested striatum homologue (Vc, Vd). The effect of stress varied with the body condition, such that the fish with better body condition had higher dopaminergic activity (stress×K; table 3). Males displayed higher dopaminergic activity than females, a trend that increased in heavier individuals (sex×body mass; table 3).

(c) Hypothalamus

We could not separate killifish preoptic and paraventricular nuclei when using a 300 µm punch needle—the minimum volume for obtaining detectable monoamine and metabolite levels. Hence, the data reported here reflect both areas combined in one punch volume. We did not analyse the remainder of the hypothalamus. We did not observe significant parasite effects on monoaminergic activity in this brain region. Serotonergic activity increased in stressed fish (table 2). The dopaminergic activity was not significantly affected by any factors, although significant interactions suggest potential effects of stress, sex, body mass and condition. Stress caused males to exhibit

higher dopaminergic activity than stressed females (stress×sex; table 3). The effect of body mass on dopaminergic activity depended on the body condition, where the dopaminergic activity increased with better condition in small fish but decreased with better condition in large fish (body mass×K).

(d) Raphe nuclei

Increasing parasite density was associated with decreased serotonergic activity in experimentally infected fish (table 2). The regression analysis revealed this trend to be driven by stressed, experimentally infected fish (figure 3), although unstressed individuals also showed significant parasite density dependence ($p=0.04$). Stress also caused a significant increase in serotonergic activity in both uninfected and experimentally infected groups (figure 2). The effect of stress also varied with body size, so that large fish exhibited increased serotonergic activity when stressed (stress×body mass; table 2). In contrast to the results on serotonergic activity, higher parasite density was associated with increased dopaminergic activity in experimentally infected fish (table 3). There was no significant relationship between metacercarial density and raphe dopaminergic activity ($p=0.1$, 0.3 for unstressed and stressed groups, respectively). The effect of stress on dopaminergic activity varied according to sex. Dopaminergic activity decreased in response to stress in females but not males (stress×sex; table 3).

Interestingly, experimentally infected fish showed drastic changes in raphe monoaminergic activity (decreased 5-HT and increased DA), despite relatively low infection intensities. This suggests that a relatively small number of parasites were able to exert a powerful effect in this brain region. One explanation could be that

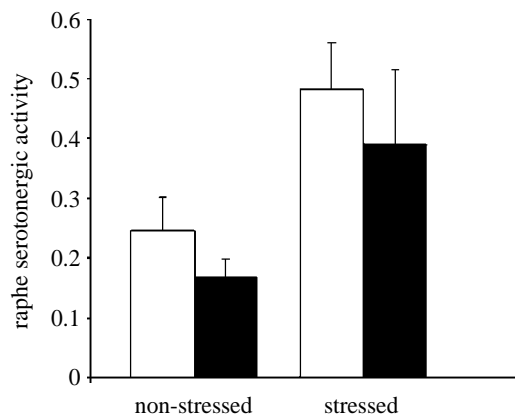


Figure 2. Killifish raphe serotonergic activity (5-HIAA/5-HT) is significantly higher in stressed individuals (open bars, uninfected; filled bars, experimentally infected; $F_{1,35} = 136.6$, $p < 0.0001$; see table 2 for full model).

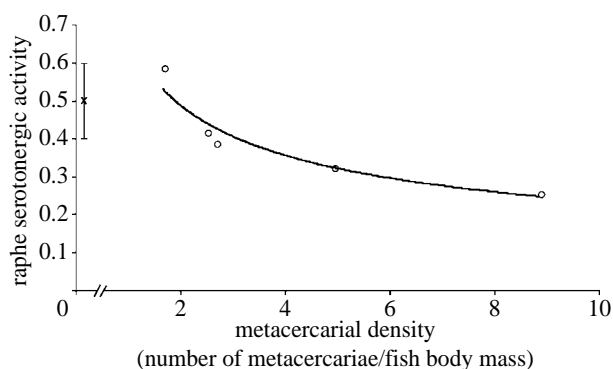


Figure 3. Regression of raphe serotonergic activity (5-HIAA/5-HT) in stressed, experimentally infected killifish against *E. californiensis* density. Means for stressed uninfected (0.5 ± 0.01 , $n = 13$) are shown for comparison only and not included in regression (circles, experimentally infected; cross, uninfected (mean ± 1 s.d.); $y = 0.672 \times^{-0.4637}$; $R^2 = 0.9434$; $p = 0.009$).

the majority of metacercariae in experimentally exposed fish were found concentrated near the brainstem. Of the total metacercariae found in the experimentally infected fish, an average of 52 per cent were found on the brainstem (rhombencephalon), 41 per cent on the cerebellum and 18 per cent on the optic lobes (mesencephalon). Only one experimentally infected fish had metacercariae located on the telencephalon (5%; regions defined in Wullimann *et al.* 1996). We were unable to compare these data with those of naturally infected fish, whose brains are usually covered completely by hundreds to thousands of metacercariae.

(e) Plasma cortisol

Cortisol increased in stressed killifish ($F_{3,36} = 68.2$, $R^2 = 0.9$, $p < 0.0001$; figure 4), verifying our stress treatment. There was no significant parasite effect on the cortisol levels. Full model details are provided in electronic supplementary material, §3.

(f) Gonadosomatic and hepatosomatic indices

There was no significant effect of metacercarial density on GSI for males ($F_{1,27} = 0.8$, $R^2 = 0.03$, $p = 0.4$; table 1) or females ($F_{1,9} = 3.4$, $R^2 = 0.3$, $p = 0.1$; table 1). However, metacercarial density did significantly affect HSI. The HSI of infected females decreased as parasite density increased ($F_{1,8} = 6.6$, $R^2 = 0.5$, $p = 0.04$; table 1), while the HSI of

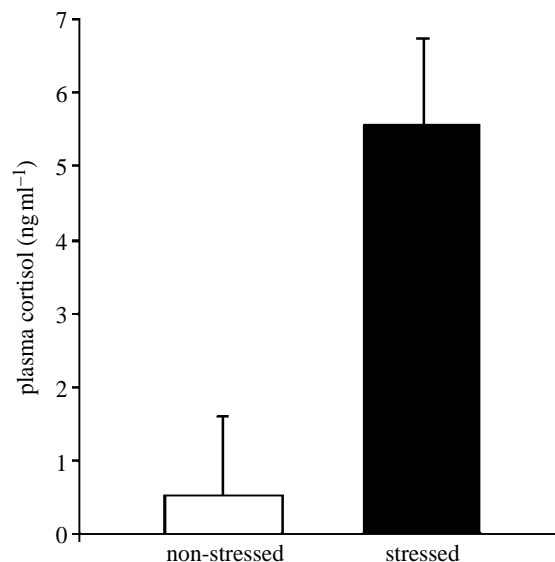


Figure 4. Plasma cortisol in stressed ($n = 20$) and non-stressed ($n = 32$) killifish ($F_{1,36} = 141.4$, $p < 0.0001$). Values are adjusted (least-squares) means that correct for the effects of infection, sex and body condition (full GLM details in electronic supplementary material, §3). Error bars represent $+1$ s.d.

infected males increased with a corresponding increase in parasite density ($F_{1,27} = 4.8$, $R^2 = 0.2$, $p = 0.04$; table 1).

4. DISCUSSION

Increasing density of *E. californiensis* correlated with altered monoaminergic activity in the killifish hippocampus, hypothalamus and raphe nuclei, despite the fact that our experimental infections resulted in parasite intensities much lower than those found in naturally occurring infections. Trematodes can adapt locally to host genotypes from the same locale (Ballabeni & Ward 1993), perhaps explaining in part why our experimental infections resulted in low intensities. Nevertheless, experimentally infected fish still exhibited strong parasite density-dependent changes.

The serotonergic profiles of stressed, uninfected killifish were consistent with those of other stressed teleosts (Winberg & Nilsson 1993). However, experimentally infected fish differed by exhibiting a parasite density-dependent decrease in raphe serotonergic activity—a reaction opposite to those of most stressed teleosts (figure 2). It seems plausible then that a parasite-induced suppression of this innate stress response could lead to different behaviour during times of stress, say, in the presence of avian predators.

Behavioural observations were not conducted in the present study owing to logistical difficulties, and therefore it cannot be concluded that altered brain monoaminergic activity would produce the exact conspicuous swimming behaviours described in Lafferty & Morris (1996). Nevertheless, the lowered serotonergic activity of experimentally infected killifish could still constitute an underlying mechanism mediating parasite-induced behavioural changes. In specific regions of the brain, serotonin (5-HT) inhibits locomotion, aggression and dominance in fishes and other vertebrates (Winberg & Nilsson 1993; Weiger 1997; but see Rueter *et al.* 1997 and Øverli *et al.* 2004 for exceptions). Several studies confirm this by

reporting the lowest levels of 5-HT levels when swimming activity is the highest (Fenwick 1970; Fingerman 1976; Øverli *et al.* 1998). Increased brain serotonergic activity is a general physiological response to stress in fishes and other vertebrates (Winberg & Nilsson 1993; Chaouloff 2000; Summers *et al.* 2005). The resultant movement inhibition, or 'freezing' (preferably under cover), is thought to be a primary avoidance response to danger in many fish species (Höglund *et al.* 2005). The raphe nuclei contain one of the largest populations of serotonin-producing neurons that project extensively throughout the brain and spinal cord (excluding the cerebellum in teleost fishes; Parent *et al.* 1984; Butler & Hodos 2005). In addition, this brain region is involved in inhibiting aggression (Summers & Winberg 2006). Therefore, we expected the raphe to reflect any changes in 5-HT activity, e.g. the initial rise in serotonergic activity, characteristic of stressed teleosts (Winberg & Nilsson 1993). The strongest effect of *E. californiensis* was a density-dependent suppression of this natural stress response in the raphe, where parasites accounted for more than 90 per cent of the variation in decreased serotonergic activity (figure 3). Their resulting influence could cause increased locomotion by affecting mid- and forebrain targets responsible for coordinating motor output. Metacercariae aggregated primarily on or near the brainstem in our low intensity, experimental infections. Hendrickson (1979) reported that brain-encysting cercariae migrate along spinal nerves and encounter the brainstem first, which would facilitate encystment near the raphe. It is possible that *E. californiensis* capitalized on its circumstances, evolving a way to affect host behaviour given its initial default location.

In contrast to the general effects of 5-HT, DA stimulates locomotory, aggressive and dominant behaviours in fishes, reptiles and mammals (Winberg & Nilsson 1993; Mok & Munro 1998; Korzan *et al.* 2006). Dopaminergic activity in the raphe nuclei regulates midbrain serotonergic activity (Korzan *et al.* 2001; Aman *et al.* 2007), whereas serotonergic raphe neurons also project back to stimulate midbrain DA-producing nuclei, forming a reciprocal feedback loop (Gervais & Rouillard 2000; Korzan *et al.* 2001; Alex & Pehek 2007). Dopaminergic activity in the raphe can also inhibit serotonergic neurons that ascend to the midbrain (Ferré *et al.* 1994). Given this, it is difficult to make definitive conclusions about the effects of increased raphe dopaminergic activity on killifish behaviour without the knowledge of the specific monoamine receptors involved.

In summary, our study provides an initial look into which neurotransmitter systems may be affected by the brain parasite *E. californiensis*. Øverli *et al.* (2001) demonstrated heightened serotonergic activity in sticklebacks infected with larval tapeworms but realized that those results could have been due to chronic stress from infection by a potentially debilitating parasite. Here, we simultaneously examined parasites and stress on killifish monoaminergic activity to disentangle their respective impacts, and we showed that *E. californiensis* exerted effects separate from those of stress. This pattern was the clearest in the raphe nuclei, where parasites inhibited the stress-induced increase in serotonergic activity in a density-dependent manner. It is unlikely that pathology or general debilitation contributed to any parasite

density-dependent effect, particularly since overall body condition (K) alone seldom explained significant variation in the monoaminergic activity. The fact that parasite density did not negatively affect HSI in both sexes suggests that the observed differences may reflect differing energetic demands between sexes, e.g. those required to develop ovaries, rather than resulting directly from parasitic infection.

The exact physiological mechanism of how *E. californiensis* alters killifish monoaminergic activity—and thereby behaviour—has yet to be determined. We know that *E. californiensis* metacercariae secrete fibroblast growth factors on the surface of their cysts *in vitro*, which helps them aggregate (J. La Clair & K. D. Lafferty, unpublished data). It seems plausible then that metacercariae could also secrete chemicals that ultimately modify killifish monoamine activity.

All animal care and research was conducted in accordance with the policies of the UCSB Institutional Animal Care and Use Committee.

The authors would like to thank Kenneth J. Renner for the HPLC and protein analysis, Jessica Cinkornpumin and Amber Kaplan for their assistance and Ryan Hechinger for his intellectual contribution. R. Hechinger and Valerie McKenzie provided their helpful comments on earlier drafts of the manuscript. The University of California Natural Reserve System provided access to field sites at Carpinteria Salt Marsh. Funding provided by the NSF/NIH Ecology of Infectious Diseases Program (DEB-0224565). Any use of trade, product or firm names in this publication is for descriptive purposes only and does not imply endorsement by the US government.

REFERENCES

- Adamo, S. A. 2002 Modulating the modulators: parasites, neuromodulators and host behavioral change. *Brain Behav. Evol.* **60**, 370–377. (doi:10.1159/000067790)
- Adamo, S. A. & Shoemaker, K. L. 2000 Effects of parasitism on the octopamine content of the central nervous system of *Manduca sexta*: a possible mechanism underlying host behavioural change. *Can. J. Zool.* **78**, 1580–1587. (doi:10.1139/cjz-78-9-1580)
- Alex, K. D. & Pehek, E. A. 2007 Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol. Ther.* **113**, 296–320. (doi:10.1016/j.pharmthera.2006.08.004)
- Allen, L. G., Yoklavich, M. M., Cailliet, G. M. & Horn, M. H. 2006 Bays and estuaries. In *The ecology of marine fishes: California and adjacent waters* (eds L. G. Allen, D. J. Pondella & M. H. Horn), pp. 119–148. Berkeley, CA: University of California Press.
- Aman, T. K., Shen, R. Y. & Haj-Dahmane, S. 2007 D-2-like dopamine receptors depolarize dorsal raphe serotonin neurons through the activation of nonselective cationic conductance. *J. Pharmacol. Exp. Ther.* **320**, 376–385. (doi:10.1124/jpet.106.111690)
- Anken, R. & Bourrat, F. 1998 *Brain atlas of the Medakafish Oryzias latipes*. Paris: INRA Editions.
- Bakker, T. C. M., Mazzi, D. & Zala, S. 1997 Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. *Ecology* **78**, 1098–1104. (doi:10.1890/0012-9658(1997)078[1098:PICIBA]2.0.CO;2)
- Ballabeni, P. & Ward, P. I. 1993 Local adaptation of the trematode *Diplostomum phoxini* to the European minnow *Phoxinus phoxinus*, its 2nd intermediate host. *Funct. Ecol.* **7**, 84–90. (doi:10.2307/2389870)

- Barber, I., Hoare, D. & Krause, J. 2000 Effects of parasites on fish behaviour: a review and evolutionary perspective. *Rev. Fish Biol. Fish.* **10**, 131–165. (doi:10.1023/A:1016658224470)
- Beckage, N. E. 1993 Endocrine and neuroendocrine host-parasite relationships. *Receptor* **3**, 233–245.
- Benjamini, Y. & Hochberg, Y. 1995 Controlling the false discovery rate—a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* **57**, 289–300.
- Berdoy, M., Webster, J. P. & Macdonald, D. W. 2000 Fatal attraction in rats infected with *Toxoplasma gondii*. *Proc. R. Soc. B* **267**, 1591–1594. (doi:10.1098/rspb.2000.1182)
- Biron, D. G., Marche, L., Ponton, F., Loxdale, H. D., Galeotti, N., Renault, L., Joly, C. & Thomas, F. 2005 Behavioural manipulation in a grasshopper harbouring hairworm: a proteomics approach. *Proc. R. Soc. B* **272**, 2117–2126. (doi:10.1098/rspb.2005.3213)
- Brassard, P., Rau, M. E. & Curtis, M. A. 1982 Parasite-induced susceptibility to predation in diplostomiasis. *Parasitology* **85**, 495–501.
- Butler, A. B. & Hodos, W. 2005 *Comparative vertebrate neuroanatomy: evolution and adaptation*. New York, NY: Wiley-Liss.
- Carney, W. P. 1969 Behavioral and morphological changes in carpenter ants harboring microcoelid metacercaria. *Am. Midl. Nat.* **82**, 605–611. (doi:10.2307/2423801)
- Chaouloff, F. 2000 Serotonin, stress and corticoids. *J. Psychopharmacol.* **14**, 139–151.
- Clements, S. & Schreck, C. B. 2004 Evidence that GABA mediates dopaminergic and serotonergic pathways associated with locomotor activity in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Behav. Neurosci.* **118**, 191–198. (doi:10.1037/0735-7044.118.1.191)
- Combes, C. 2001 *Parasitism: the ecology and evolutions of intimate interactions*. Chicago, IL: The University of Chicago Press.
- de Jong-Brink, M., Bergamin-Sassen, M. & Soto, M. S. 2001 Multiple strategies of schistosomes to meet their requirements in the intermediate snail host. *Parasitology* **123**, S129–S141. (doi:10.1017/S0031182001008149)
- Fabre-Nys, C. 1998 Steroid control of monoamines in relation to sexual behaviour. *Rev. Reprod.* **3**, 31–41. (doi:10.1530/ror.0.0030031)
- Fenwick, J. C. 1970 Brain serotonin and swimming activity in goldfish, *Carassius auratus*. *Comp. Biochem. Physiol.* **32**, 803–806. (doi:10.1016/0010-406X(70)90830-3)
- Ferré, S., Cortes, R. & Artigas, F. 1994 Dopaminergic regulation of the serotonergic raphe-striatal pathway—microdialysis studies in freely moving rats. *J. Neurosci.* **14**, 4839–4846.
- Fingerman, S. W. 1976 Circadian rhythms of brain 5-hydroxytryptamine and swimming activity in teleost, *Fundulus grandis*. *Comp. Biochem. Phys. C* **54**, 49–53. (doi:10.1016/0306-4492(76)90024-1)
- Gervais, J. & Rouillard, C. 2000 Dorsal raphe stimulation differentially modulates dopaminergic neurons in the ventral tegmental area and substantia nigra. *Synapse* **35**, 281–291. (doi:10.1002/(SICI)1098-2396(20000315)35:4<281::AID-SYN6>3.0.CO;2-A)
- Haas, W., Wulff, C., Grabe, K., Meyer, V. & Haeberlein, S. 2007 Navigation within host tissues: cues for orientation of *Diplostomum spathaceum* (Trematoda) in fish towards veins, head and eye. *Parasitology* **134**, 1013–1023. (doi:10.1017/S0031182007002430)
- Hechinger, R. F., Lafferty, K. D., Huspeni, T. C., Brooks, A. & Kuris, A. M. 2007 Can parasites be indicators of free-living diversity? Relationships between species richness and the abundance of larval trematodes and of local fishes and benthos. *Oecologia* **151**, 82–92. (doi:10.1007/s00442-006-0568-z)
- Helluy, S. & Thomas, F. 2003 Effects of *Microphallus papillorobustus* (Platyhelminthes: Trematoda) on serotonergic immunoreactivity and neuronal architecture in the brain of *Gammarus insensibilis* (Crustacea: Amphipoda). *Proc. R. Soc. B* **270**, 563–568. (doi:10.1098/rspb.2002.2264)
- Hendrickson, G. L. 1979 *Ornithodiplostomum ptychocheilus*: migration to the brain of the fish intermediate host, *Pimephales promelas*. *Exp. Parasitol.* **48**, 245–258. (doi:10.1016/0014-4894(79)90106-1)
- Höglund, E., Kolm, N. & Winberg, S. 2001 Stress-induced changes in brain serotonergic activity, plasma cortisol and aggressive behavior in Arctic charr (*Salvelinus alpinus*) is counteracted by L-DOPA. *Physiol. Behav.* **74**, 381–389. (doi:10.1016/S0031-9384(01)00571-6)
- Höglund, E., Weltzien, F. A., Schjolden, J., Winberg, S., Ursin, H. & Doving, K. B. 2005 Avoidance behavior and brain monoamines in fish. *Brain Res.* **1032**, 104–110. (doi:10.1016/j.brainres.2004.10.050)
- Holmes, J. C. & Bethel, W. M. 1972 Modification of intermediate host behaviour by parasites. In *Behavioural aspects of parasite transmission* (eds E. U. Canning & C. A. Wright), pp. 123–150. New York, NY: Academic Press Inc.
- Hoogenboom, I. & Dijkstra, C. 1987 *Sarcocystis cernae*—a parasite increasing the risk of predation of its intermediate host, *Microtus arvalis*. *Oecologia* **74**, 86–92. (doi:10.1007/BF00377350)
- James, M. D., Hole, D. R. & Wilson, C. A. 1989 Differential involvement of 5-hydroxytryptamine (5HT) in specific hypothalamic areas in the mediation of steroid-induced changes in gonadotropin release and sexual behavior in female rats. *Neuroendocrinology* **49**, 561–569. (doi:10.1159/000125169)
- Kavaliers, M., Colwell, D. D. & Choleris, E. 1999 Parasites and behavior: an ethopharmacological analysis and biomedical implications. *Neurosci. Biobehav. Rev.* **23**, 1037–1045. (doi:10.1016/S0149-7634(99)00035-4)
- Klein, S. L. 2003 Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiol. Behav.* **79**, 441–449. (doi:10.1016/S0031-9384(03)00163-X)
- Knudsen, R., Gabler, H. M., Kuris, A. M. & Amundsen, P. A. 2001 Selective predation on parasitized prey—a comparison between two helminth species with different life-history strategies. *J. Parasitol.* **87**, 941–945.
- Korzan, W. J., Summers, T. R. & Summers, C. H. 2000 Monoaminergic activities of limbic regions are elevated during aggression: influence of sympathetic social signaling. *Brain Res.* **870**, 170–178. (doi:10.1016/S0006-8993(00)02420-3)
- Korzan, W. J., Summers, T. R., Ronan, P. J., Renner, K. J. & Summers, C. H. 2001 The role of monoaminergic nuclei during aggression and sympathetic social signaling. *Brain Behav. Evol.* **57**, 317–327. (doi:10.1159/000047250)
- Korzan, W. J., Forster, G. L., Watt, M. J. & Summers, C. H. 2006 Dopaminergic activity modulation via aggression, status, and a visual social signal. *Behav. Neurosci.* **120**, 93–102. (doi:10.1037/0735-7044.120.1.93)
- Kuris, A. 2003 Evolutionary ecology of trophically transmitted parasites. *J. Parasitol.* **89**, s96–s100.
- Lafferty, K. D. 1992 Foraging on prey that are modified by parasites. *Am. Nat.* **140**, 854–867. (doi:10.1086/285444)
- Lafferty, K. D. 1999 The evolution of trophic transmission. *Parasitol. Today* **15**, 111–115. (doi:10.1016/S0169-4758(99)01397-6)
- Lafferty, K. D. & Morris, A. K. 1996 Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology* **77**, 1390–1397. (doi:10.2307/2265536)

- Libersat, F. & Pflueger, H. J. 2004 Monoamines and the orchestration of behavior. *Bioscience* **54**, 17–25. (doi:10.1641/0006-3568(2004)054[0017:MATOOB]2.0.CO;2)
- Martin, W. E. 1950 *Euhaplorchis californiensis* N.G., N. SP., Heterophyidae, Trematoda, with notes on its life cycle. *Trans. Am. Microsc. Soc.* **69**, 194–209. (doi:10.2307/3223410)
- Martin, W. E. 1955 Seasonal infections of the snail *Cerithidea californica* Haldeman, with larval trematodes. In *Essays in the natural sciences in honor of Captain Allan Hancock*, pp. 203–210. Los Angeles, CA: University of Southern California Press.
- McCurdy, D. G., Forbes, M. R. & Boates, J. S. 1999 Evidence that the parasitic nematode *Skrjabinoclava* manipulates host *Corophium* behavior to increase transmission to the sandpiper, *Calidris pusilla*. *Behav. Ecol.* **10**, 351–357. (doi:10.1093/beheco/10.4.351)
- McNeff, L. L. 1978 Marine cercariae from *Cerithidea pliculosa* Menke from Dauphin Island, Alabama; Life Cycles of Heterophyid and Opisthorchiid Digenea from *Cerithidea* Swainson from the Eastern Gulf of Mexico. MSc thesis, University of Alabama, USA.
- Mok, E. Y. M. & Munro, A. D. 1998 Effects of dopaminergic drugs on locomotor activity in teleost fish of the genus *Oreochromis* (Cichlidae): involvement of the telencephalon. *Physiol. Behav.* **64**, 227–234. (doi:10.1016/S0031-9384(98)00038-9)
- Moore, J. 2002 *Parasites and the behavior of animals. Oxford series in ecology and evolution*. New York, NY: Oxford University Press, Inc.
- Northcutt, R. G. 1983 Telencephalic organization in ray-finned fishes. In *Fish neurobiology* (eds R. E. Davis & R. G. Northcutt), pp. 203–236. Ann Arbor, MI: The University of Michigan Press.
- Øverli, Ø., Winberg, S., Damsgard, B. & Jobling, M. 1998 Food intake and spontaneous swimming activity in Arctic char (*Salvelinus alpinus*): role of brain serotonergic activity and social interactions. *Can. J. Zool.* **76**, 1366–1370. (doi:10.1139/cjz-76-7-1366)
- Øverli, Ø., Harris, C. A. & Winberg, S. 1999 Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain Behav. Evol.* **54**, 263–275. (doi:10.1159/000006627)
- Øverli, Ø., Pall, M., Borg, B., Jobling, M. & Winberg, S. 2001 Effects of *Schistocephalus solidus* infection on brain monoaminergic activity in female three-spined sticklebacks *Gasterosteus aculeatus*. *Proc. R. Soc. B* **268**, 1411–1415. (doi:10.1098/rspb.2001.1668)
- Øverli, Ø., Korzan, W. J., Larson, E. T., Winberg, S., Lepage, O., Pottinger, T. G., Renner, K. J. & Summers, C. H. 2004 Behavioral and neuroendocrine correlates of displaced aggression in trout. *Horm. Behav.* **45**, 324–329. (doi:10.1016/j.yhbeh.2004.01.001)
- Parent, A. 1983 The monoamine-containing neuronal systems in the teleostean brain. In *Fish neurobiology* (eds R. E. Davis & R. G. Northcutt), pp. 285–315. Ann Arbor, MI: The University of Michigan Press.
- Parent, A., Poitras, D. & Dube, L. 1984 Comparative anatomy of central monoaminergic systems. In *Handbook of chemical neuroanatomy* (eds A. Bjorklund & T. Hokfelt), pp. 409–439. Amsterdam, NY: Elsevier Science Publishers.
- Peter, R. E., Macey, M. J. & Gill, V. E. 1975 Stereotaxic atlas and technique for forebrain nuclei of killifish, *Fundulus heteroclitus*. *J. Comp. Neurol.* **159**, 103–127. (doi:10.1002/cne.901590107)
- Renner, K. J. & Luine, V. N. 1984 Determination of monoamines in brain nuclei by high-performance liquid-chromatography with electrochemical detection—youth vs middle-aged rats. *Life Sci.* **34**, 2193–2199. (doi:10.1016/0024-3205(84)90320-5)
- Rojas, J. M. & Ojeda, F. P. 2005 Altered dopamine levels induced by the parasite *Profilicollis antarcticus* on its intermediate host, the crab *Hemigrapsus crenulatus*. *Biol. Res.* **38**, 259–266.
- Rosenberg, L. A., Pfluger, H. J., Wegener, G. & Libersat, F. 2006 Wasp venom injected into the prey's brain modulates thoracic identified monoaminergic neurons. *J. Neurobiol.* **66**, 155–168. (doi:10.1002/neu.20203)
- Rueter, L. E., Fornal, C. A. & Jacobs, B. L. 1997 A critical review of 5-HT brain microdialysis and behavior. *Rev. Neurosci.* **8**, 117–137.
- Salas, C., Broglio, C. & Rodriguez, F. 2003 Evolution of forebrain and spatial cognition in vertebrates: conservation across diversity. *Brain Behav. Evol.* **62**, 72–82. (doi:10.1159/000072438)
- Sandland, G. J. & Goater, C. P. 2000 Development and intensity dependence of *Ornithodiplostomum pychocheilus* metacercariae in fathead minnows (*Pimephales promelas*). *J. Parasitol.* **86**, 1056–1060. (doi:10.1645/0022-3395(2000)086[1056:DAIDOO]2.0.CO;2)
- Shannon, N. J., Gunnet, J. W. & Moore, K. E. 1986 A comparison of biochemical indices of 5-hydroxytryptaminergic neuronal activity following electrical stimulation of the dorsal raphe nucleus. *J. Neurochem.* **47**, 958–965.
- Shaw, J. C. 2007 Neural mechanisms of behavior modification in killifish (*Fundulus parvipinnis*) by a brain parasite (*Euhaplorchis californiensis*) and the ecology of the host-parasite relationship. PhD Dissertation, University of California, Santa Barbara, CA, USA.
- Shaw, J. C., Aguirre-Macedo, L. & Lafferty, K. D. 2005 An efficient strategy to estimate intensity and prevalence: sampling metacercariae in fishes. *J. Parasitol.* **91**, 515–521. (doi:10.1645/GE-385R2)
- Skalova, A., Kodym, P., Frynta, D. & Flegr, J. 2006 The role of dopamine in *Toxoplasma*-induced behavioural alterations in mice: an ethological and ethopharmacological study. *Parasitology* **133**, 525–535. (doi:10.1017/S0031182006000886)
- Stibbs, H. H. 1985 Changes in brain concentrations of catecholamines and indoleamines in *Toxoplasma gondii* infected mice. *Ann. Trop. Med. Parasitol.* **79**, 153–157.
- Summers, C. H. & Winberg, S. 2006 Interactions between the neural regulation of stress and aggression. *J. Exp. Biol.* **209**, 4581–4589. (doi:10.1242/jeb.02565)
- Summers, C. H. *et al.* 2005 Does serotonin influence aggression? Comparing regional activity before and during social interaction. *Physiol. Biochem. Zool.* **78**, 679–694. (doi:10.1086/432139)
- Tain, L., Perrot-Minnot, M. J. & Cezilly, F. 2006 Altered host behaviour and brain serotonergic activity caused by acanthocephalans: evidence for specificity. *Proc. R. Soc. B* **273**, 3039–3045. (doi:10.1098/rspb.2006.3618)
- Thomas, F. & Poulin, R. 1998 Manipulation of a mollusc by a trophically transmitted parasite: convergent evolution or phylogenetic inheritance? *Parasitology* **116**, 431–436. (doi:10.1017/S003118209800239X)
- Thomas, F., Ulitsky, P., Augier, R., Dusticier, N., Samuel, D., Strambi, C., Biron, D. G. & Cayre, M. 2003 Biochemical and histological changes in the brain of the cricket *Nemobius sylvestris* infected by the manipulative parasite *Paragordius tricuspidatus* (Nematomorpha). *Int. J. Parasitol.* **33**, 435–443. (doi:10.1016/S0020-7519(03)00014-6)
- Thomas, F., Adamo, S. & Moore, J. 2005 Parasitic manipulation: where are we and where should we go? *Behav. Process.* **68**, 185–199. (doi:10.1016/j.beproc.2004.06.010)

- Vyas, A., Kim, S.-K., Giacomini, N., Boothroyd, J. C. & Sapolsky, R. M. 2007 Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proc. Natl Acad. Sci. USA* **104**, 6442–6447. (doi:10.1073/pnas.0608310104)
- Watt, M. J., Forster, G. L., Korzan, W. J., Renner, K. J. & Summers, C. H. 2007 Rapid neuroendocrine responses evoked at the onset of social challenge. *Physiol. Behav.* **90**, 567–575. (doi:10.1016/j.physbeh.2006.11.006)
- Weiger, W. A. 1997 Serotonergic modulation of behaviour: a phylogenetic overview. *Biol. Rev. Camb. Philos. Soc.* **72**, 61–95. (doi:10.1017/S0006323196004975)
- West, J. M. & Zedler, J. B. 2000 Marsh-creek connectivity: fish use of a tidal salt marsh in southern California. *Estuaries* **23**, 699–710. (doi:10.2307/1352896)
- Winberg, S. & Nilsson, G. E. 1993 Roles of brain monoamine neurotransmitters in agonistic behavior and stress reactions, with particular reference to fish. *Comp. Biochem. Phys. C* **106**, 597–614. (doi:10.1016/0742-8413(93)90216-8)
- Winberg, S., Myrberg, A. A. & Nilsson, G. E. 1993 Predator exposure alters brain serotonin metabolism in bicolor damselfish. *Neuroreport* **4**, 399–402. (doi:10.1097/00001756-199304000-00014)
- Wullimann, M. F. & Mueller, T. 2004 Teleostean and mammalian forebrains contrasted: evidence from genes to behavior. *J. Comp. Neurol.* **475**, 143–162. (doi:10.1002/cne.20183)
- Wullimann, M. F., Rupp, B. & Reichert, H. 1996 *Neuroanatomy of the zebrafish brain: a topological atlas*. Basel; Boston, MA: Birkhäuser Verlag.
- Yoshino, T. P. 1971 Helminth Parasitism in the Pacific Killifish *Fundulus parvipinnis* from Goleta Slough. Dissertation, University of California, Santa Barbara, California, MA.